INTRODUCTION

Preterm is defined as babies born alive before 37 weeks of pregnancy are completed ***(Blencowe et al., 2012).***

Preterm birth complications are estimated to be responsible for 35% of the world's 3·1 million annual neonatal deaths and are now the second most common cause of death after pneumonia in children under 5 years old ***(Liu et al., 2012).*** Preterm birth also increases the risk of death due to other causes, especially from neonatal infections ***(Lawn et al., 2010).***

Lactoferrin (LF) is secreted by epithelial cells into exocrine fluids: seminal fluid, pancreatic exocrine secretions, tears, saliva, uterine secretions, and milk.

Neutrophils also secrete LF locally at sites of inflammation. Levels vary greatly, with the highest concentration in mammalian milk. Milk from mothers who deliver preterm may differ from those delivering at termand contain less human LF ***(Mehta and Petrova, 2011).***

The risk for invasive fungal infections is high in very low birth weight (VLBW) infants (< 1500 g) and highest for infants born at the youngest gestational ages who survive past the immediate postnatal period. ***The incidence of IFIs increases in these infants due to many factors such as;***

- Invasive procedure, such as central vascular catheters and endotracheal tube.
- Exposure to broad spectrum antibiotics and parentral nutrition.
- The occasional use of postnatal steroids and gastric acid inhibitors

(Kaufman, 2010)

Probiotics are microorganisms that are believed to provide health benefits when consumed ***(Hill et al., 2014).***

That mean the dependence of the intestinal microbes on the food makes it possible to adopt measures to modify the flora in our bodies and to replace the harmful microbes by useful microbes ***(Rijkers et al., 2011).***

An example is lactealfort probiotic which is a unique in that it is the only heat killed, freeze-dried lyophilized. acidophilus, L. Lactobacillus in dose of 6 x 109 colony forming units (CFU) divided into 2doses/day till reaching full feed ***(Collee et al., 1996).***

Invasive fungal infections (IFIs) represent an increasing cause of severe morbidity and mortality in most neonatal intensive care units (NICUs) ***(Wynn et al., 2012).***

LF is involved in innate immunity mechanisms with several documented anti-infective properties, including

antifungal activity, This last mechanism is related to both fungistatic effects, and the activity of the N-terminal, 11 aminoacidic peptide of LF called lactoferricin [*hLF(1-11*)] ***(Orsi, 2004).***

Both bovine and human LF shares a high structural homology, as well as the same antimicrobial properties ***(Wynn et al., 2012).***

AIM OF THE WORK

To evaluate the efficacy of enteral lactoferrin in combination with the probiotic species (Lactobacillus Delbrueckii and Lactobacillus Fermentum) in the prevention of invasive fungal infections in preterm infants admitted in (NICUs).

Chapter One
LACTOFERRIN OVERVIEW

Lactoferrin (Lf) known as ***lactotransferrin (LTF***), which is a multifunctional protein of the transferrin family.

Lf is a globular glycoprotein with a molecular mass of about 80 kDa that is widely represented in various secretory fluids, such as milk, saliva, tears, and nasal secretions. Lf is also present in secondary granules of polymorph nucleus and is secreted by some acinar cells. Lf can be purified from milk or produced recombinantly. Human colostrum (*"first milk"*) has the highest concentration, followed by human milk, then cow milk (150 mg/L) **(*Levin et al., 2006).***

At least 60 gene sequences of LF have been characterized in 11 species of mammals. In most species, stop codon is TAA and TGA in the nucleus. Deletions, insertions and mutations of stop codons affect the coding part and its length varies between 2, 055 and 2, 190 nucleotide pairs.

Gene polymorphism between species is much more diverse than the intra specific polymorphism of LF. This variation may indicate functional differences between different types of LF ***(Kang et al., 2008).***

The saturated iron concentration in LF in human milk is estimated as 10 to 30% (100% corresponds to all LF molecules containing 2 iron atoms).

It is demonstrated that LF is involved not only in the transport of iron, zinc and copper, but also in the regulation of their intake. Presence of loose ions of zinc and copper does not affect the iron binding ability of LF, and might even increase it ***(Shongwe et al., 2001).***

Lactoferrin and immunity

LF is one of the components of the immune system of the body; it has antimicrobial activity (bacteriocide, fungicide) and is part of the innate defense, mainly at mucosa. In particular, LF provides antibacterial activity to infants ***(Levin et al., 2006).***

Given the potential beneficial actions of LF in a range of inflammatory and infectious conditions, several trials of LF supplementation have been undertaken over the past 10 years ***(Ochoa et al., 2012).***

Although recombinant human LF is available, it remains very expensive. Most trials have assessed the effect of bovine LF (processed from cows' milk) which is 70% homologous with human LF but has higher antimicrobial activity, is inexpensive, and is available commercially as a food supplement in a stable powder form. Bovine LF has been a component of the human infant diet for thousands of years and is registered 'generally recognised as safe', Federal Drug

Administration with no reports of human or animal toxicity ***(Yamauchi et al., 2000).***

Furthermore, because bovine LF does not bind strongly to the LF receptor in the human small intestine, it is not absorbed via the gastrointestinal tract and does not generate hypersensitivity or allergic immunological reactions ***(Hochwallner et al., 2010).***

LF is digested by the enzymes of stomach, predominantly by pepsin and the resultant secondary product of digestion is known as lactoferricin (LFcin), it has more potent antimicrobial activity than parent molecule ***(Valenti and Antonini, 2005).***

LF belongs to the innate immune system. Apart from its main biological function, namely binding and transport of iron ions, LF also has antibacterial, antiviral, antiparasitic, catalytic, anti-cancer, and anti-allergic functions and properties ***(Adlerova et al., 2008).***

LF also has prebiotic properties, creating an enteric environment for the growth of beneficial bacteria and reducing colonisation with pathogenic species ***(Tian et al., 2010).***

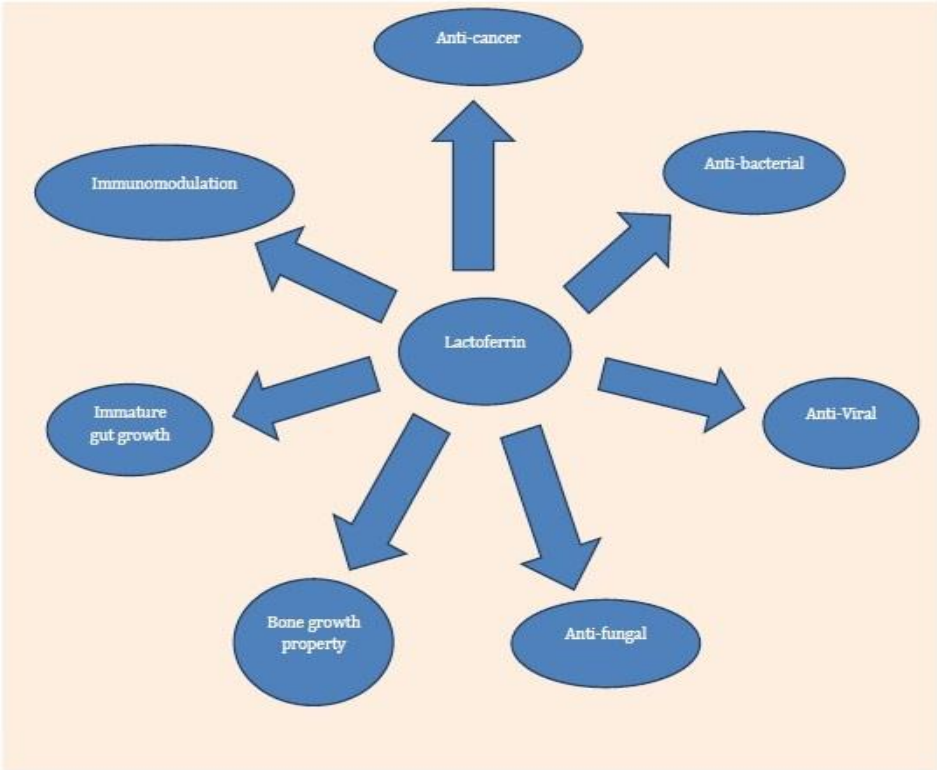

Figure (1): Showing spectrum of lactoferrine ***(Rawat et al., 2012).***

Antifungal actions of lactoferrine

Lactoferrin and lactoferricin inhibit *in vitro* growth of *Trichophyton Mentagrophytes*, which are responsible for several skin diseases such as ringworm ***(Wakabayashi et al., 2000).***

LF also acts against the *Candida albicans* – a diploid fungus (a form of yeast) that causes opportunistic oral and genital infections in humans. Fluconazole has long been used against *Candida albicans*, which resulted in emergence of strains resistant to this drug. However, a combination of

lactoferrin with fluconazole can act against fluconazole-resistant strains of *Candida albicans* as well as other types of Candida: *C. glabrata, C. krusei, C. parapsilosis* and *C. tropicalis.*

In particular, synthetic peptide 1–11 lactoferricin shows much greater activity against *Candida albicans* than native lactoferricin ***(Lupetti et al., 2003).***

Administration of LF through drinking water to mice with weakened immune systems and symptoms of aphthous ulcer reduced the number of *Candida albicans* strains in the mouth and the size of the damaged areas in the tongue **(*Takakura et al., 2003).***

Oral administration of LF to animals also reduced the number of pathogenic organisms in the tissues close to the gastrointestinal tract. *Candida albicans* could also be completely eradicated with a mixture containing LF, lysozyme and itraconazole in HIV-positive patients who were resistant to other antifungal drugs ***(Masci, 2000).***

Such antifungal action when other drugs deem inefficient is characteristic of LF and is especially valuable for HIV-infected patients ***(Kuipers et al., 1999).***

Contrary to the antiviral and antibacterial actions of LF, very little is known about the mechanism of its antifungal action.

LF seems to bind the plasma membrane of *C. albicans* inducing an apoptotic-like process ***(Andrés et al., 2008).***

LF is a broad-spectrum antimicrobial peptide against bacteria, fungi, viruses, and protozoa, and shows potent synergism with fluconazole in azole –susceptible isolates of Candida albicans obtained from neonates with sepsis ***(Venkatesh et al., 2008).***

This synergism might also be applicable against refractory candidiasis. Also showed that a combination of fluconazole with LF related compounds exerted synergistic effects on cell growth, even in the case of azole-resistant C. albicans ***(Wakabayashi et al., 1998),*** but it has not yet been elucidated exactly how combination with LF will influence effectiveness of different drugs against various resistance mechanisms.

Candida. spp. are known to acquire azole resistance by at least three different mechanisms: altered sterol synthetic pathway from native ergosterol due to ERG3 mutation, resulting in the production of non-toxic alternative sterol in the presence of azoles ***(Miyazaki et al., 1999),*** decreased substrate affinity due to mutations in the target molecule, 14-alpha-demetlylase, which is encoded by ERG11**,** and decreased intracellular concentrations of drugs due to over expression of genes encoding efflux pumps, such as CDR1 and Ca MDR ***(Vanden Bossche et al., 2000).***

Antibacterial activity

Lactoferrin's primary role is to sequester free iron, and in doing so remove essential substrate required for bacterial growth.

Antibacterial action of lactoferrin is also explained by the presence of specific receptors on the cell surface of microorganisms, Lactoferrin binds to lipopolysaccharide of bacterial walls, and the oxidized iron part of the lactoferrin oxidizes bacteria via formation of peroxides, This affects the membrane permeability and results in the cell breakdown (lysis) ***(Farnaud and Evans, 2003).***

Although lactoferrin also has other antibacterial mechanisms not related to iron, such as stimulation of phagocytosis, the interaction with the outer bacterial membrane described above is the most dominant and most studied ***(Xanthou, 1998).***

Lactoferrin not only disrupts the membrane, but even penetrates into the cell. Its binding to the bacteria wall is associated with the specific peptide lactoferricin, which is located at the N-lobe of lactoferrin and is produced by *in vitro* cleavage of lactoferrin with another protein, trypsin ***(Sojar et al., 1998).***

A mechanism of the antimicrobial action of lactoferrin has been reported as lactoferrin targets H^+-ATPase and

interferes with proton translocation in the cell membrane, resulting in a lethal effect *in vitro* ***(Andrés and Fierro, 2010).***

Lactoferrin prevents the attachment of *H. pylori* in the stomach, which in turn, aids in reducing digestive system disorders.

Bovine lactoferrin has more activity against *H. pylori* than human lactoferrin ***(Prescriber's letter, 2007).***

Antiviral activity

Lactoferrin acts, mostly *in vitro*, on a wide range of human and animal viruses based on DNA and RNA genomes ***(van der Strate et al., 2001)*** including the herpes simplex virus 1 and 2, cytomegalovirus, HIV, hepatitis C virus ***(Giansanti et al., 2002),*** hantaviruses, rotaviruses, poliovirus type 1, ***(Azzam et al., 2007)*** human respiratory syncytial virus, murine leukemia viruses and Mayaro virus ***(Carvalho et al., 2014).***

The most studied mechanism of antiviral activity of lactoferrin is its diversion of virus particles from the target cells. Many viruses tend to bind to the lipoproteins of the cell membranes and then penetrate into the cell ***(Nozaki et al., 2003).***

Lactoferrin binds to the same lipoproteins thereby repelling the virus particles. Iron-free apolactoferrin is more efficient in this function than hololactoferrin; and lactoferricin,

which is responsible for antimicrobial properties of lactoferrin, shows almost no antiviral activity ***(van der Strate et al., 2001).***

Beside interacting with the cell membrane, lactoferrin also directly binds to viral particles, such as the hepatitis viruses ***(Nozaki et al., 2003).***

This mechanism is also confirmed by the antiviral activity of lactoferrin against rotaviruses ***(Sojar et al., 1998)*** which act on different cell types.

Lactoferrin also suppresses virus replication after the virus penetrated into the cell ***(Puddu et al., 1998).*** Such an indirect antiviral effect is achieved by affecting natural killer cells, granulocytesand macrophages – cells, which play a crucial role in the early stages of viral infections, such as severe acute respiratory syndrome (SARS) ***(Reghunathan et al., 2005).***

Anticarcinogenic activity

The anticancer activity of (bLF) has been demonstrated in experimental lung, bladder, tongue, colon, and liver carcino geneses, possibly by suppression of phase I enzymes, such as cytochrome P450 1A2 (CYP1A2) ***(Tsuda et al., 2002).***

Also, in another experiment done on hamsters, bovine lactoferrin decreased the incidence of oral cancer by 50% ***(Chandra Mohan et al., 2006).***

Because bLF by far did not show any toxicity and because it's readily available in milk, bLF offers promise as a potential chemopreventive agent for oral cancer. Currently, bLF is used as an ingredient in yogurt, chewing gums, infant formulas, and cosmetics.

Mechanism of action of lactoferrin

- ***Microbicidal actions of lactoferrin***

  A. Disruption of cell membrane

  B. Iron sequestration

  C. Prevention of biofilm formation

  D. Proteolysis of virulence factors

  E. Blocks bacterial adhesion to host cells by binding to glycosaminoglycans

  F. Initiates "anoikis" in which cells containing viable bacteria undergo apoptosis

  G. Enhances the growth of the normal commensal bifidogenic microflora in the gut.

- ***Anti-cancer action***

  A. Cell cycle arrest.

  B. Promotes apoptosis.

  C. Anti-angiogenesis.

  D. Antimetastasis.

  E. Immune modulation.

  F. Promotes necrosis.

(Lonnerdal, 2003)

Table (1): Spectrum of variuos effect of lactoferrin.

Anti-microbial	
Antibacterial	**Anti-fungal**
1. *Pseudomonas aeruginosa*	1. Candida Albicans
2. *Haemophilusinfluenzae*	2. yeast
3. *E coli*	
4. *Helicobacter pylori*	
5. *Clostridium difficile*	
6. *Shigella Flexnieria*	
7. *Staphylococcus aureus*	
8. *Streptococcus Mutans*	
9. *Streptococcus Pneumoniae*	
10. *Aggregatibacter actinomycetemcomitans*	
11. *Yersinia enterocolitica*	
12. *Listeria monocytogenes*	
Antiviral	**Anti-cancer**
1. *Human Immunodeficiency Virus*	1. Head and neck squamous cell carcinoma
2. *Cytomegalovirus (CMV),*	2. Breast cancer
3. *Herpes simplex virus (HSV),*	3. Colon carcinoma
4. *Hepatitis C virus (HCV),*	4. Malignant melanoma
5. *Rotavirus,*	5. bronchogenic carcinoma
6. *Poliovirus(PV),*	
7. *Respiratory syncytial virus (RSV)*	
8. *Hepatitis B virus (HBV),*	
9. *Parainfluenza virus (PIV),*	
10. *Alphavirus,*	
11. *Hantavirus,*	
12. *Human papillomavirus (HPV),*	
13. *Feline calicivirus (FCV),*	
14. *Adenovirus,*	
15. *Enterovirus 71 (EV71),*	
16. *Echovirus 6,*	
17. *Influenza A virus,*	
18. *Japanese encephalitis viru*	
19. Tomato yellow leaf curl virus (TYLCV***)***	

(Pandita et al., 2015)