

Effect of Enteral Lactoferrin Administration on Iron Status In Neonates

Thesis

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List of Abbreviations

APCs	:	Antigen presenting cells
BCG	:	Bacille Calmette-Guerin strain of Mycobacterium bovis
BLf	:	Bovine lactoferrin
BPD	:	Broncho-pulmonary dysplasia
CBC	:	Complete blood count
CD4	:	Cluster of differentiation 4
CD8	:	Cluster of differentiation 8
CRP	:	C-reactive protein
DMT1	:	Divalent metal transporter 1
DNA	:	Deoxyribonucleic acid
ESAs	:	Erythropoiesis stimulating agents
GMA	:	Granulocyte and monocyte adsorptive apheresis
Hb	:	Haemoglobin
HBV	:	Hepatitis-B virus
Hct	:	Hematocrit
HCV	:	Hepatitis-C virus
HIV	:	Human immunodeficiency virus
HLf	:	Human Lf
IBD	:	Inflammatory bowel disease
ID	:	Iron deficiency
IFI	:	Invasive fungal infections
IFN- γ	:	Interferon- γ
IL-18	:	Interleukin-18
LF	:	Lactoferrin
LFH	:	Lactoferrin hydrolysates
MCH	:	Mean cell hemoglobin
MCHC	:	Mean cell hemoglobin concentration

List of Abbreviations (Cont.)

MCV	:	Mean cell volume
MHC II	:	Major histocompatibility complex II
NICU	:	Neonatal intensive care unit
NK	:	Natural killer
PMNs	:	Polymorphoneuclear leukocytes
RBC	:	Red blood cell
RDW	:	Red Blood Cell Distribution Width
rHuEPO- β	:	Recombinant human erythropoietin- β
ROP	:	Retinopathy of prematurity
ROS	:	Reactive oxygen species
SF	:	Serum ferritin concentration
TBI	:	Transferrin-bound iron
Tf	:	Transferrin
TfR1	:	Serum transferrin receptor 1
TfR1	:	Transferrin receptor 1
Th1	:	T-helper cell type-1
U937	:	Human monocytic cells
UC	:	Ulcerative colitis
VLBW	:	Very low birth weight
WHO	:	The World Health Organization

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Introduction

Iron deficiency is the most common nutritional deficiency in infants and children. The World Health Organization (WHO) estimates that anemia affects one-quarter of the world's population and is concentrated within newborns, preschool-aged children and women; a majority of the anemia is due to iron deficiency (*Righetti et al., 2012*) (*World Health Organization, Geneva, 2008*) .

Iron deficiency anemia develops when body stores of iron drop too low to support normal red blood cell (RBC) production. Inadequate dietary iron, impaired iron absorption, bleeding, or loss of body iron in the urine may be the cause (*Hempel and Bollard, 2016*).

The functional deficits associated with anemia include gastrointestinal disturbances and impaired cognitive function, immune function, exercise or work performance, and body temperature regulation (*Clark, 2008*).

In infants and children, iron deficiency anemia can result in psychomotor and cognitive abnormalities that, without treatment, can lead to learning difficulties (*Tawfik et al., 2015*).

Lactoferrin (LF) is an iron-binding glycoprotein found in the milk of mammals such as humans, a large amount of LF is found especially in the colostrums, and its content differs among animal species. In humans, the LF content in foremilk is 5 to 7 g/l, almost 10 times higher than that in

cow's milk (0.8 g/l). In addition, LF is also found in tears, saliva, and neutrophils, and is considered to be one of defensive factors that prevent bacterial and viral infection (*Koikawa et al., 2008*).

It has also been found that the iron-binding capacity of LF is about 300-fold greater than that of transferrin. It has been confirmed that LF regulates iron absorption. Recently, it was reported that oral LF increases hemoglobin and total serum iron in pregnant women and neonates (*Rosa et al., 2017*).

It decrease rate of packed RBCS transfusion and its hazards in neonates and enhance serum hemoglobin and ferritin level during Nadir period in both preterm and full term infants (*Kohorn and Ehrenkranz, 2009*).

As matter of fact, in anemic subjects also including non-pregnant women, Lactoferrin oral administration firstly increases the concentration of total serum iron indicating an efficient restoring of ferroportin function (*Paesano et al., 2009*).

Furthermore, the increase of serum hepcidin (or prohepcidin), related to an increase of hematological parameters, should be consider as signal of a regulatory mechanism to avoid an excess of iron export from cells to blood, subsequent to the restoring of ferroportin expression by LF. Therefore, the efficacy of LF in curing Iron deficiency anemia can be explained through its influence on systemic iron homeostasis (*Paesano et al., 2010*).

Aim of the work

Our primary aim is:

To evaluate the effect of enteral lactoferrin supplementation on levels of haemoglobin, haematocrit and serum levels of ferritin in infants admitted in the neonatal intensive care unit.

Our secondary aim is:

To study the effect of lactoferrin on the need and the frequency of blood transfusion in NICU, feeding intolerance, time to reach full enteral intake, weight gain, signs of sepsis, the length of hospital stay and the mortality as well as to study the relation between milk type and the serum ferritin.

Chapter one:

"Lactoferrin"

1.Introduction:

Milk proteins are considered to be the most important source of bioactive peptides (*Nagpal et al., 2011*).

Lactoferrin (Lf) belongs to the transferrin (Tf) family and is a non-heme iron binding glycoprotein with molecular weight of 78 kDa that contains around 690 amino acid residues (*Kanwar et al., 2015*).

It is found in bovine milk as well as in humans, In human it is one of the major proteins of all exocrine secretions including saliva, tears, semen, vaginal fluids, gastrointestinal fluids, nasal mucosa and bronchial mucosa (*Iigo et al., 2009*).

Breast milk represents the main source of Lf found in the gut of infants and high levels of fecal Lf in in the first days of life represents the initiation, development and/or composition of the neonatal gut microbiota (*Mastromarino et al., 2014*).

Lf is also known for its anti-bacterial, antifungal, antiviral, antimicrobial, anti-oxidant, anti-inflammatory, anti-parasitic, anti-allergic and most importantly anticancerous properties (*Kanwar et al., 2015*).

The highest concentration of lactoferrin is found in human colostrum and then human milk followed by cow

milk, and it is the second most abundant milk protein after Caseins (*Ballard and Morrow, 2013*).

The iron binding affinity of Lf is known to be the maximum amongst transferrin family. Lf can remain bound to iron in varying pH range (*Sill et al., 2016*).

The major role of Lf in humans is the transportation of iron in blood plasma. Lactoferrin, in its natural form, is partially saturated with iron and hence can be fully saturated with iron from the external environment (*Mills et al., 2010*).

2. Structure:

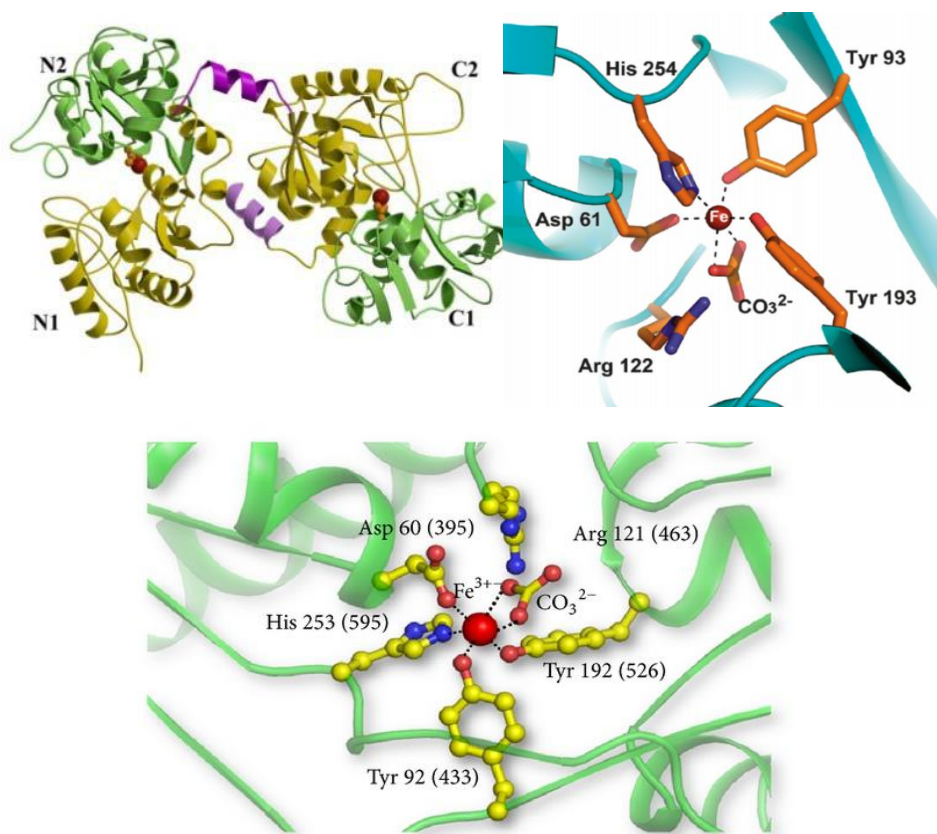


Fig. (1): The structure of Lf. (Naidu, 2002).

The structure of Lf consists of a single polypeptide chain containing about 690 amino acids folded into two globular lobes. These lobes are highly homologous to each other and are connected by an α -helical residue providing flexibility to the Lf molecule. Each lobe consists of two domains forming a single iron binding site, allowing each Lf molecule to strongly bind two ferric ions (*Latorre et al., 2010*).

The open, iron-free form of Lf is known as apo-Lf while the closed, iron-rich form is holo-Lf, which differs in tertiary structure and is more resistant to proteolysis than apo-Lf (*Fernandes and Carter, 2017*).

In addition to binding Iron, Lf has also been observed to bind a range of other compounds including lipopolysaccharides, heparin, Deoxyribonucleic acid (DNA), and metal ions including Copper, Zinc, and Manganese (*Porcheron et al., 2013*).

3. Functions, Uses and Applications of lactoferrin :

There are two known types of lactoferrin "The Natural Form or Human Lactoferrin And The Bovine Lactoferrin" (*Adlerova et al., 2008*).

*** Human Lactoferrin:**

Human Lf (HLf) is isolated from the colostrum by various methods including chromatographic techniques like ion exchange chromatography (*Liang et al., 2011*).

HLf was tested against a number of bacteria to observe its bactericidal activity and it was reported that Lf exhibited a

very effective response against a various range of bacteria including species of Streptococcus, Salmonella, Shigella, Staphylococcus and Enterobacter (*Bruni et al., 2016*).

HLF has also been found to play a role in the nutritional activity by increasing the thymidine content in damaged crypt cells helping in their recovery and development and is also used as a diagnostic marker as its immunochemical detection in the feces indicates the presence of gastrointestinal disorders and risk of colon cancer (*Kanwar et al., 2015*).

*** Bovine Lactoferrin:**

The concept of oral administration of bovine lactoferrin (BLf) was first introduced in 1978 when a BLf containing dry milk was marketed by the Morinaga Milk industry in Japan (*Roy et al., 2015*) (*Chierici et al., 1992*).

Later on, the research and evidence have indicated the role of orally administered BLf in the improvement in intestinal microbial flora, increased serum ferritin and hematocrit levels, reduction in lower respiratory track diseases and anti-infective activities (*Kanwar et al., 2015*).

Orally administered BLf has also shown beneficial effects in other animal infection models including oral candidiasis, influenza virus pneumonia and skin infections due to herpes virus (*El-Hafez et al., 2013*).

Enhanced production of interleukin-18 (IL-18) in intestinal epithelial cells, IL-10 and interferon- γ (IFN- γ) in intestinal intraepithelial lymphocytes and mesenteric