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"Human Milk Component (HMC) Therapy": A Novel Treatment for Gut Maturation of Preterm Infants

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Abstract

Even though the process of gut maturation starts right from intrauterine life, it really gets accelerated only after birth through enteral route. The preterm infants as well as infants with intrauterine growth restriction and those who had absent or reversed umbilical cord flow during their intrauterine stay are the candidates to have immature gut. They are also prone to the complications like feeding intolerance and necrotizing enterocolities.

It is well established that the human milk due to the presence of growth factors and cellular components, play important role in the process of gut maturation. With this background, we have hypothesised and proposed the concept of Human Milk Component (HMC) isolation and therapy for gut maturation in preterm infants. The aqueous fraction of human milk rich in growth factors and bioactive substances and the cellular fraction rich in different kinds of cells including mesenchymal stem cells are isolated. They are given through the feeding tube to the infants with immature gut.

The aqueous component of human milk acts by a passive mechanism while the cellular component acts by an active mechanism. This concept further strengthens the importance of human milk.

Keywords: Human milk; Gut maturation; Growth factors in human milk; Cells in human milk; Necrotizing enterocolitis

Introduction

In human beings organ maturation is particularly rapid during the perinatal period. Development of the Gastrointestinal Tract (GIT) is stimulated by the transition from mainly parenteral nutrition before birth (via the placenta) to exclusively enteral nutrition after birth. The ability of the mother to control the development and maturation of GIT of child is a possibility available only to mammals. Our own original study [1] has interestingly demonstrated that the levels of growth factors, especially Vascular Endothelial Growth Factor (VEGF) and Hapatocyte Growth Factor (HGF) are multifold higher in the aqueous fraction of human milk than those observed in the umbilical cord blood. This clearly indicates the physiological changeover of the source of the growth factors from the placenta to the breast milk. As the gut maturation mainly occurs rapidly after birth by enteral route, the higher levels of the growth factors in the breast milk play a very important role in the entire process. In our original study [2], we have also documented the presence of the multipotent mesenchymal stem cells in human milk. Their immunofleuroscent labeling revealed positivity for mesenchymal stem cell surface markers like CD44, CD29 and SCA-1. We have also shown their 'In vitro' differentiation into multiple lineages like adipocytes, chondrocytes, osteocytes and the islet cells of pancreas. Apart from these stem cells, the cellular fraction of breast milk contain progenitor cells, leukocytes and myoepethelial cells. Most of the leukocytes are macrophages and neutrophils which phagocytose microbial pathogens. Lymphocytes including T cells, natural killer cells and antibody producing B cells make upto 10% of leukocytes in human milk.These cells prime the gut and help in further maturation.

Presently in the field of haematology and transfusion medicine the concept of blood component therapy is well established and proved to be superior to the modality of whole blood transfusion.On the similar grounds we have developed concept of Human Milk Component (HMC) therapy,where two main components of human milk viz. aqueous fraction (rich in growth factors and other bioactive substances) and cellular fraction (rich in different types of cells) are administered to preterm infants to accelerate the gut maturity.

In this review, we are highlighting the pathophysiology of immature gut observed in preterm infants and its subsequent clinical complications. We are also discussing the role of HMC therapy which has a strong physiological basis in accelerating the gut maturity and preventing bad clinical sequel [3].The HMC acts both by active (through cellular fraction) and by passive (through bioactive substances present in aqueous fraction) mechanisms.

Physiology of gut maturation

Maturation of the fetal Gastrointestinal Tract (GIT) is influenced by both luminal stimuli (e.g. swallowed fluid) and hormonal factors (e.g. endogenous cortisol release). Hence enteral intake of nutrients in the newborns elicits structural and functional GIT changes [3,4]. Enteral nutrient intake may also play a role before birth. During the last trimester, human fetuses swallow fluid (up to 750 ml) that contains dilute concentrations of protein free amino acids and a variety of growth factors derived from amniotic fluid and lung fluid as well from as nasal secretions [5,6]. Evidence from human and experimental animals (e.g. sheep, rabbits) suggest that the fetal esophageal obstruction may impair growth of the intestine, particularly if the obstruction is complete [7-9].

Fetal GIT maturation is subject to endocrine regulation. In particular, the increase in fetal glucocorticoid secretion toward term is known to affect the functional development of the fetal liver, lungs and GIT [10-12]. Consistent with this, administration of glucocorticoids to preterm infants before and after birth reduces the risk of intestinal disease [13]. Fetal studies in both animals and human suggest that development of gastrointestinal motility begins with the second trimester, but matures in the third trimester [14]. Intestinal epithelia are joined by tight junctions that regulate the intestinal permeability and form by 10 weeks of gestation [15]. The process of fetal intestinal secretion and absorption mature under the influence of amniotic fluid from 26 weeks of gestation to full term [16]. Goblet cells are found throughout the small and large intestine. These specialized enterocytes secrete mucus forming a thick protective layer over the intestinal mucosa. This mucus layer impedes direct microbial epithelial binding and enhances removal of adherent bacteria [17]. Developmental expression of mucin genes changes throughout the intestine and matches adult pattern expression between 23 to 27 weeks of gestation [18]. Another aspect of the intestinal epithelial barrier is biochemical defense. Paneth cells, which are specialized secretory enterocytes located at the base of small intestinal crypts, secret lysozyme, phospholipase A2 and antimicrobial peptides that regulate the distribution of different bacterial populations [19]. Defensins(α and β) and cathelicidins are the two main families of antimicrobial peptides produced by intestinal cells [20].

Many growth factors (especially Epidermal Growth Factor EGF) are major tropic factors for the development of the intestine. EGF receptor has been identified on the basolateral surface of enterocytes [21]. Exogenous infusion of EGF in utero has been shown to accelerate the maturation of intestine enzyme activity as well as stimulate intestinal growth [22]. Interestingly, the salivary level of EGF is directly proportional to the gestational age of the infant [23]. Hepatocyte Growth Factor (HGF) and Vascular Endothelial Growth Factor (VEGF) are expressed in gut tissues and play a major role in the mucosal growth and repair [24]. VEGF is also expressed in the small intestine, predominantly in the lamina propria mast cells. The aqueous fraction of the human milk contains many bioactive substances like EGF, VEGF, HGF amphiregulin, epiregulin, betacellulin and neuregulin which all play a physiological role in neonatal gut growth and development [25,26]. Widdowson et al. documented the marked increase in gastrointestinal weight, length and protein as well as DNA content of colostrum fed neonatal piglets [27]. Interestingly, EGF is resistant to degradation within gastric milieu of the preterm infant which suggests that milk born EGF may retain bioactivity in the neonatal GIT [28].Another interesting observation is the evidence of higher levels of VEGF and other growth factors in the milk of mothers who have given birth to preterm infants than in the milk of the mothers who have delivered at term [29,30].

TYPE	COMPONENT	Functions
HUMORAL (Aqueous fraction)	EGF	Stimulate intestinal growth 22 Maturation of intestinal enzymes 22
	VEGF	Mucosal growth and repair 24
	HGF	Gut growth and repair 25,26
	Amphireguline	Gut growth and repair 25,26
	Epireguline	Gut growth and repair 25,26
	Neuregulin	Gut growth and repair 25,26
Cellular fraction	Macrophages	Local immunity in GIT 31
	Neutrophils	Local immunity in GIT 31
	Natural killer cells	Local immunity in GIT 31
	Antibody producing β cells	Antimicrobial peptide production 20
	Mesenchymal stem cells (MSC)	Secretion of growth factors 1,2
	Myoepethelial mammary cells	Role unproven 1

Table 1: Human Milk Components and their physiological functions

Exposure of the neonate to the bacteria during birth and immunological factors in the breast milk, promote the maturation of the infant's gut and gut associated immune system. In addition to the growth factors and other bioactive substances, the cellular fraction of the human milk plays important role in the neonatal GIT maturation process. Colostrum contains approximately 5106 cells per ml, which

comprise of macrophages, neutrophils, natural killer cells, antibody producing cells and mesenchymal stem cells. There is evidence to suggest that these cells survive passage through the infants GIT where they are absorbed and prime the GIT for the process of maturation [31].The breast milk leukocytes interact with the intestinal mucosa of the infant which could lead to form of tolerance to maternal antigens.

Thus, the gut maturation of the infant though begins from the intrauterine life, gets really accelerated only after birth through the aqueous fraction and cellular fraction of the human milk. The detailed compositions of these fractions are listed in Table 1.

Predisposing factors for immature gut

The gut of the infant represents a complex organ system with regional differences, which reflect selective digestive and absorptive functions that change constantly in response to bodily requirements. As a barrier to the external environment, gut epithelium must be renewed rapidly and repeatedly. The entire process of the fetal gut maturation accelerates with gestational age and hence preterm babies have poorly developed gut. The infants who have Intrauterine Growth Restriction (IUGR) due to various causes, (common being pregnancy induced hyper tension PIH) often have immature gut. Such infants during their intrauterine life have absent or reversed umbilical artery flow [31,32]. During their intrauterine stay the blood flow is diverted to the brain which ultimately leads to hypoxia of the gut (brain sparing effect). Perinatal asphyxia also affects the process of gut maturation [33].

Sequele of immature gut

The most common sequele of the premature gut is the development of problems like feeding intolerence and Nectrotizing Enterocolitis (NEC). Upto 90% of the infants with NEC are of low birth weight. Netrotizing enterocolitis is an inflammatory bowel disease of the neonates with significant morbidity and mortality in premature infants. Immature intestinal motility and digestion may predispose preterm infants to NEC. Studies of intestinal motility have shown that preterm infants can have immature motility patterns when compared with full term infants [34]. Similarly fetal hypoxia (as seen in IUGR or PIH) can further reduce postnatal intestinal 6motility [35]. Immature motility patterns alter normal peristaltic activity and result in over growth of anaerobic bacteria in the small intestine with malabsorption of dietary nutrients [36]. In addition to impaired intestinal motility, premature infants have not yet developed the ability to digest and absorb nutrients and incompletely digested molecules could further contribute to intestinal injury [37]. Thus, impaired digestion of nutrients coupled with delayed transit time and bacterial overgrowth could result in intestinal injury with immature host and barrier defenses. NEC affects all portions of GIT most commonly the jejunum, terminal ileum and proximal colon. The gut with NEC shows mucosal and transmucosal coagulation necrosis, haemorhage, inflammation, ulceration and reparative changes [38]. Mortality rate of NEC overall is 20-40% [39].

Role of Human Milk in the process of gut maturation in the infants

Human milk is a dynamic multifaceted fluid containing nutrients, bioactive factors and cells, required for infant health and development. Its composition varies by stage of lactation and between term and pre-term infants. The significance of human milk is established beyond doubt and hence exclusive human milk feeding for the first 6 months of life with continued breast feeding for 1 to 2 years of life or longer is recognized as normative standard for infant feeding [40].

Preterm infants fed with Human Milk (HM) display improved feeding tolerance [41] and are less colonized by pathogenic organisms [42].They develop fewer severe infections [43]. They experience decreased lengths of hospital state [44] and reduced rates of readmission after discharge [45]. A prospective, nonrandomized study showed NEC to be 6 to 10 times more common among formula fed babies and 3 times more common among formula plus human milk, compared to those who were fed on human milk alone [46].Hence, early enteral feeding in preterm and Very Low Birth Weight (VLBW) infants is associated with reduced incidence of sepsis, improved milk tolerance and accelerated post natal growth [47].

Human Milk Composition and its impact on gut maturation

A medline search using only the phrase "human milk composition" reveals a steady increase in publications since 1960 with new components still being identified in human milk. The contents of colostrum are so useful for the early and late development of infant that it is called as "Liquid Gold". The important components of human milk are macronutrients, bioactive substances and cellular fraction. It provides 65 -70 kcal/dl and are highly correlated with the fat content.

Bioactive components are present in the aqueous fraction of human milk. They are defined as the elements that "affect biological processes or substrates and hence have an impact on body function and ultimately health" [48]. Bioactive components in human being come from variety of sources, some are produced and secreted by mammary epithelium while others are drawn from maternal serum and carried across the mammary epithelium by receptor mediated transport. Growth factors especially Epidermal Growth Factor (EGF) present in human milk is critical in gut maturation and healing of intestinal mucosa. The average level of EGF in colostrum is 2000 fold higher than in maternal serum [49]. Interestingly, preterm milk contains higher EGF than term milk [50]. The immaturity of the new born intestine extends to enteral nervous system which requires Brain Derived Neurotropic Factors (BDNF) and Glial cell-line Derived Neurotropic Factors (GDNF) for its development [51]. Both these factors are present in aqueous fraction of human milk. The other growth factors like Insulin like Growth Factors (IGF) which stimulates erythropoesis and increases hematocrit [52] while Vascular Endothelial Growth Factor (VEGF) helps to reduce the burden of Retinopathy of Prematurity (ROP) [53].

The cellular fraction of human milk contains various types of cells including mesechymal stem cells. Thus human milk is not "merely nutrition". Rather it contains variety of factors with medicinal qualities that have a profound role in infant survival and health.

Concept of human milk component (HMC) therapy

The concept of blood component therapy is well established. It is avoiding the transfusion of unnecessary blood components while providing only essential components like platelets or cryoprecipitate or various other combinations. On the similar lines, we have evolved the concept of human milk component isolation and therapy. The purpose of such isolation is to provide aqueous fraction of human milk rich in bioactive substances and immune globulins along with cellular fraction both of which play immense role in the acceleration of gut maturation which is essential in preterm or IUGR infant with

immature gut. The fat component is eliminated to avoid digestive load on immature gut, as the main aim is to enhance and repair gut than providing calorie which is provided by parenteral route at initial stage of premature infants.

Protocol of HMC separation

The breast milk is collected by taking aseptic precaution with manual expression in the clean sterile singly packed sterile polypropylene 10 ml test tubes (falcon labware). The milk volume requirement is decided by the weight of the baby (30ml/kg).The tubes are tightly capped and are immediately transported to the tissue culture laboratory at room temperature.

The separation of the human milk components is done in a laminar flow in class 100 atmosphere maintained additionally by Air Handling Unit (AHU).The tubes were centrifuged at a 1500RPM for 20 minutes. After centrifugation, three layers are detected in the test tube viz. the cell pellet at the bottom, clear aqueous fraction in the middle and yellow thick layer of the fat at the top. The upper thick layer of the fat is discarded. The aqueous layer is filtered through 0.22µ filter (Millipore) prior to use.

Thus, HMC in the form of tubes of aqueous fraction and a tube with cell fraction are safely isolated. The final volume is adjusted to the dosage of 20ml/kg of the baby weight.

The initial experience of HMC therapy for gut maturation in preterm infants

A prospective interventional observational study was carried at Patki Research Foundation and Hospital and at Masai Institute of children and Research centre in collaboration with D Y. Patil medical university at Kolhapur, India, during the period of April 2013 to March 2014. The study was approved by the Institutional ethics committee and Institutional Review Board. The methods and purpose of the study were explained to the parents and their informed written consent was obtained prior to enrolment.

The preterm infants with gestational age between 30 to 34 weeks with birth weight above 1 Kg and postnatal age between 4 days to 20 days were included. Exclusion criteria included the following: Birth weight less than 1 kg, gestational age above 35 weeks, infants with major congenital anomalies or chromosomal disorders and HIV reactive mothers. The preterm neonates with or without IUGR, having diagnosed with necrotizing enterocolitis who are in stage IA as per modified Bell's criteria with systemic signs in the form of temperature instability and /or apnea and/or bradycardia along with abdominal signs like abdominal distension and/or elevated pregavage residuals and/or occult blood in stool were selected for HMC therapy. All these selected subjects were given 20ml/kg of human milk cellular and aqueous fraction via feeding tube in three devided doses over a 24 hrs period where cell fraction was given along with first dose of aqueous fraction.

All these neonates were continued on other supportive care. After the therapy all these neonates were monitored by meticulous recording of vital parameters as well as by retesting occult blood in stool on day 3 of therapy. Blood count and CRP was repeated after 48hrs and 96 hrs after HMC therapy. Details of clinical data are given in Table 2.

Results

HMC therapy was given to 20 subjects. Table 2 shows the clinical data of the study subject. Out of the 20 mothers, 7 had mild Pregnancy Induced Hypertension (PIH). The 7 neonates of the mothers who had PIH had element of Intra Uterine Growth Restriction (IUGR).Initial Septic screen was negative in all these infants. 8 neonates were requiring inotropes in the form of dopamine 8–12 µg/kg/min.

Sl. No	Parameters	values
1	Maternal age	30.5 + 1.84 years
2	Gestational age of infants	32.6 + 1.35 weeks
3	Sex of infants M/F	13/7
4	Birth weight	1.56 + 0.38 kg
5	Mode if delivery(caesarean/vaginal birth)	12/8
6	APGAR score at 1 and 5 min	7.6 and 8.4
7	NEC diagnosed on (day of life)	6 + 2 days

Table 2: Clinical data

9 neonates required surfactant administration for respiratory distress syndrome (RDS) and were on minimal oxygen support with nasal prong at the time of intervention. All the study subjects were on minimal enteral feeds by gavage at the time of diagnosis of NEC where 60% of neonates were receiving expressed breast milk. 80% (n=16) neonates tolerated human milk components (HMC) therapy without any gastrointestinal side effects, where as other 4 showed mild worsening 1of distension and episodes of vomiting which lasted for 24hrs but by 72hrs of therapy all these neonates were stable and were considered for restarting tropical feeds. After restarting the feeds on day 3rd of therapy 70% (n=14) of subjects had good tolerance until the time of discharge whereas remaining had intermittent episodes of feed intolerance which was managed by feeding by continuous slow gavage feeding. On an average by day 7 of starting feeds, those who tolerated HMC well (80%) were on full feeds. Subsequent follow up for 2 months showed growth and development as per established standards, all the study subjects' blood parameters were normal and stool occult blood was negative on day 3 of therapy.

Conclusion

The gut of the preterm infant has several deficits right from the poor neuromuscular and epithelial development to the reduced physiological process of motility and absorption. Additionally, those preterm infants with IUGR are more vulnerable to the diseases of immature gut like feeding intolerance and Necrotizing Enterocolits (NEC). Human Milk Component (HMC) therapy sounds more physiological as it provides aqueous fraction rich in bioactive substances and cellular fraction rich in different kinds of cells, both of which are essential for priming and accelerating maturation process of the gut as well as for the repair of damaged premature gut.

In certain clinical situations, especially mothers who have delivered very premature infant often have inadequate milk secretions. In such situations the aqueous fraction of pasteurised donor human milk (human milk bank) can be used. The cellular fraction of infant's own mother milk can be supplemented to aqueous fraction. Even though the process of pasteurisation often reduces the potency of the growth factors, it is not entirely lost.

The multicentric controlled double blinded studies with large number of subjects are necessary to strengthen the concept.HMC can also be considered as an alternative good option to enteral feeds of expressed breast milk especially in preterm infants and in infant having IUGR and early sepsis.

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