
**“INCIDENCE OF NECROTIZING ENTEROCOLITIS
AMONG PRETERM NEONATES RECEIVING
PASTEURIZED DONOR HUMAN MILK – A ONE YEAR
CROSS SECTIONAL STUDY AT KLE DR.PRABHAKAR
KORE HOSPITAL AND MRC, BELAGAVI”.**

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ACCEPTANCE LETTER

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LIST OF ABBREVIATIONS

^o F	-	Degree Fahereinheit
ABG	-	Arterial blood gas
APSA	-	American Pediatric Surgical Association
CFT	-	Capillary refilling time
CI	-	Confidence interval
cm	-	Centimetre
CRH	-	Corticotrophin releasing hormone
CRP	-	C-reactive protein
cumm	-	Cubic millimetre
DHM	-	Donor human milk
DIC	-	Disseminated intravascular coagulation
DNA	-	Deoxyribonucleic acid
EBF	-	Exclusive breast feeding
EPO	-	Erythropoietin
ER	-	Endoplasmic reticulum
FFP	-	Fresh Frozen plasma
g	-	Grams
GAs	-	Gestational ages
gm	-	Grams
gm%	-	Gram percent
HHM	-	High human milk
HIV	-	Human immunodeficiency virus
HM	-	Human milk
HMB	-	Human milk bank

HMO	-	Human milk oligosaccharides
HsCRP	-	Highly sensitive C-reactive protein
Hsp70	-	Heat shock protein 70
I-FABP	-	Intestinal fatty acid-binding protein
IgA	-	Immunoglobulin A
IL	-	Interleukin
IQR	-	Interquartile range
IUD	-	Intrauterine death
Kg	-	Kilograms
KMC	-	Kangaroo mother care
LBW	-	Low birth weight
LHM	-	Low human milk
LOS	-	Late onset sepsis
LSCS	-	Lower segment caesarean section
MAS	-	Meconium aspiration syndrome
MDP	-	Muramyl dipeptide
min	-	Minutes
mL	-	Milliliter
MOM	-	Mothers own milk
n	-	Total number
NEC	-	Necrotizing enterocolitis
NMR	-	Neonatal Mortality Rate
NG	-	Nasogastric tube
NICU	-	Neonatal intensive care unit
NST	-	Nonstress test

NVD	-	Normal vaginal delivery
OG	-	Orogastric
OR	-	Odds ratio
p	-	Probability
PCV	-	Packed cell volume
PDHM	-	Pasteurized donor human milk
PIH	-	Pregnancy induced hypertension
PPROM	-	Preterm premature rupture of the membranes
PRBC	-	Packed Red Blood Cells
RCTs	-	Randomized controlled trial
RDP	-	Randomized donor platelet
rEPO	-	Recombinant human erythropoietin
RR	-	Relative risk
SD	-	Standard deviation
TLR4	-	Toll - like receptor 4
TNEC	-	Transfusion-associated necrotizing enterocolitis
UK	-	United Kingdom
UP	-	Uttar Pradesh
VLBW	-	Very low birth weight
vs	-	Versus
WBC	-	White blood cell count
WHO	-	World Health Organization
wk	-	Week

ABSTRACT

Background and objectives

This study was conducted to find out the incidence of Necrotizing enterocolitis (NEC) among preterm neonates receiving Pasteurized donor human milk (PDHM), to assess acceptability of PDHM in Recipients and to evaluate the outcomes with respect to incidence of sepsis, mortality rate, duration of NICU stay, growth parameters.

Methodology

The cross-sectional study was conducted from January 2019 to February 2020 in the Department of Pediatrics, KLES Dr.PrabhakarKore Hospital and Medical Research Centre, Belagavi. During the study period a total of 1854 neonates were admitted in NICU among them, 634 were preterms. Of this, 433 preterm neonates were offered PDHM as other 201 were on breastfeed. Out of 433 preterms; 312 accepted PDHM which were enrolled in study of which 91 were excluded as per exclusion criteria and data pertaining to 221 neonates was available for analysis.

Results

Our study reported that the Incidence of NEC was significantly low among the neonates who received the PDHM (4.07%). Acceptability of PDHM was high in the study population (72.06%) with parent refusal on social factors and fear of transmission of disease were the cause for non-acceptability. Sepsis was noted in 32 (21.62%) neonates. Of the 32 neonates with sepsis, 26 (81.25%) had fungal sepsis and 6 (18.75%) had bacterial sepsis. Significant increase in weight (4.61%), height (3.15%) and head circumference was (4.07%) noted at discharge ($p < 0.001$).

Conclusion

The present study showed low incidence of NEC in preterm neonates receiving PDHM. Acceptability of PDHM was high in the study population. Sepsis was low with no mortality in neonates receiving PDHM. There was a positive effect of PDHM on growth and breastfeeding at discharge.

Keywords: Necrotising enterocolitis; Preterm birth; Pasteurized donor human milk; Sepsis.

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INTRODUCTION

Globally preterm birth rate is 5-18%; In India 13% and 16% in North-Karnataka.¹ Worldwide the Neonatal mortality rate (NMR) is 18/1000 live births, in India 25 and in Karnataka 23/1000 live births, of which 43.7% are due to preterm and its complications.² Of the complications, in the preterm infants Necrotizing enterocolitis (NEC) and sepsis are the two most important causes of neonatal mortality.

Necrotizing enterocolitis (NEC) is the most common and most serious gastrointestinal emergency in the newborn infant .It is pre-dominantly a disease of the preterm infant and about 10% of cases can occur in term babies. It is an acute inflammatory injury of the distal small and often proximal large intestine. Surgical pathology reveals segmental coagulative necrosis of the mucosa with focal haemorrhage as evidence for Ischemia.³

The Incidence of NEC varies between 0.3 to 2.4% in every 1000 live births.³ In most centres , the incidence of NEC is between 1 to 5% of all neonatal intensive care unit (NICU) admissions and 5-10% of very low birth weight (VLBW) infants (<1500 gm). Overall NEC is responsible for 12 % of deaths in extreme premature infants <27 weeks of gestation.⁴

The risk factors for the development of NEC include prematurity, bacterial dysbiosis and formula feeding. The triad of intestinal ischemia (injury), enteral nutrition (metabolic substrate) and bacterial translocation has classically been linked to NEC. The commonest risk factor stated in the literature for NEC is prematurity.⁴

At present , NEC is thought to develop in the premature babies in the setting of bacterial colonization , often after administration of non-breast milk feeds, as it interacts with the increased reactivity of the premature intestinal mucosa leading to mucosal destruction and impaired mesentric perfusion along with increased expression of the bacterial receptor Toll - like receptor 4 (TLR4) in the premature gut ; it plays a crucial role in the regulation of normal intestinal development through its effect on Notch signalling pathway.⁵

Human milk protects premature infants from NEC and sepsis; as it has both nutritional and anti - infective properties .Factors include lysozyme, secretory Immunoglobulin A (IgA), lactoferrin, bile salt-stimulating lipase, growth factors, cytokines, vitamin E, glutathione; Components such as epidermal growth factor, nucleotides, and glutamine also stimulate intestinal maturity; Human milk oligosaccharides (HMO) are prebiotic agents which act by enhancing proliferation of beneficial bifidobacterial species and preventing the adhesion of pathogenic bacteria to the intestinal epithelium.^{6,7}

Most of the mothers of preterm neonates are unable to produce adequate milk in initial days. Under these circumstances when mothers own milk (MOM) is not available, WHO recommends pasteurized donor human milk as the next best alternative especially for LBW babies.⁸ Pasteurized donor human milk (PDHM) is distributed through Human milk bank which systematically collects; pasteurizes, stores and distribute the milk. There are meta-analysis and systemic reviews among PDHM and non-breast milk feeds which have shown lower incidence of NEC, may be due to supply of immuno-protective factors to the immature mucosa.⁹⁻¹¹ Narayan et al.¹² showed reduction in infection being 10.5 % with PDHM which was much lesser

compared to 33.3% with formula feeds,¹² there are systemic reviews and RCT's which have shown lesser incidence of feeding intolerance and bronchopulmonary dysplasia in neonates receiving PDHM compared to formula feeds.¹³ Considering these facts, and to help prevent NEC and late onset sepsis (LOS) our hospital has taken a leap forward by opening a Human milk bank (Amrutha) in March 2018 and since then PDHM is being provided to the babies admitted to the NICU.

There is limited data regarding PDHM and NEC in Indian context. In view of this; this cross - sectional study was planned to find out the incidence of NEC among preterm neonates receiving PDHM and to assess acceptability of PDHM in recipients and to evaluate the outcomes among preterm neonates receiving PDHM with respect to sepsis, mortality rate, duration of NICU stay, growth parameters (weight, length, head circumference) and time of initiation of breastfeeding.

OBJECTIVES

The objectives of this study were;

Primary

To find out the Incidence of NEC among preterm neonates receiving pasteurized donor human milk.

Secondary

- To assess acceptability of PDHM in Recipients.
- To evaluate the outcomes among preterm neonates receiving PDHM
 - Incidence of sepsis
 - Mortality rate
 - Duration of NICU stay
 - Growth parameters – weight gain, length, head circumference.
 - Time of initiation of breastfeeding.

REVIEW OF LITERATURE

Necrotizing enterocolitis (NEC) is the commonest and most serious gastrointestinal emergency in the newborn infant. It is marked by variable intestinal injury from epithelial injury to transmural involvement and perforation. It is often marked by inflammation and bacterial invasion.¹⁵

Background

Charles Billard described a case nearly 200 years ago in Paris, France from the Hospital des infants trouves in which ‘a neonate developed a swollen tense abdomen with greenish then bloody diarrhea, bradycardia, cold extremities, and subsequent death.’ The autopsy confirmed inflamed terminal ileum, with friable mucosa and the surface covered with blood. The mucosa was very soft that it ‘turned to mash when scraped with the fingernail’. The above mentioned features are compatible with clinical findings that are being observed in neonates having NEC now, thus being the first reported case of NEC.¹⁶

In 1944, Heinrich Willi reported 62 cases of ‘malignant enteritis’ in neonates, two-third had birth weights < 2500 grams, associated with overcrowding in the nursery, and they typically occurred in clusters. These findings may be the first crediting what later on was reported as a ‘NEC epidemic’, which are rare nowadays.¹⁶

In 1952 Schmidt and Kaiser described 85 cases , in two of its reports where neonates were having tense and swollen abdomen , blood in the stools, with pathological evidence of ulcerated and necrotic bowel which was termed ‘enterocolitis ulcerosa necroticans’.^{16,17}

Arthur Steinen radiologist from Michigan in 1951 noticed pneumatosis intestinalis in the radiograph of a neonate having bloody stools, and described as ‘from the mesenteric root gas can dissect, extend to the mesenteric insertion of the intestine and from here either dissect along the subserosal layers or, following the blood vessels...enter the submucosa’. This finding become the radiologic hallmark of NEC.¹⁶

Now more and more preterm neonates are able to survive because of the advancement in the technique of care and management of these neonates, it is found that hypoxia is the important factor other than enteral feeding, intestinal microbial flora (gram negative bacteria) and inflammation leading to development of intestinal injury in preterm neonates, However the impact of hypoxia and mesentric ischemia leading to NEC remains controversial. Prematurity being single most important risk factor, probably due to immature and impaired host defense.¹⁶

Bell’s criteria was proposed in 1978 to classify NEC. The severity of NEC was described on clinical and radiographic signs and helps in early assessment. However the disease understanding has improved which lead to modification of the criteria’s, yet there continues to be controversy about the validity of this staging system at lower gestational ages (GAs). Severity of NEC plays a key role in both the management and the outcome of affected neonates. Neonates, categorized as Bell’s stage II and III, respectively, are at higher risk of developing peritonitis, sepsis, bowel perforation, and other severe complications including capillary leak syndrome and multi-system organ failure.¹⁸⁻²¹

Epidemiology

Worldwide

The current occurrence of NEC is in fact a manifestation of the tremendous success achieved by neonatologists in their ability to keep premature infants alive at ever earlier gestational ages accounting for 11% of live births worldwide. In the USA alone, the rate of prematurity is about 10% of all births, with rates as high as 13.23% in black individuals of non-Hispanic origin. The European studies determined the incidence of NEC to be up to 13% among infants born 33 weeks of gestation or whose birth weight is 2,500 g.²²⁻²⁶

Higher incidence is observed in male babies of African American descent than in any other single demographic, which could be related to the higher incidence of prematurity.²²

Mostly disease onset occurs between 27–34 weeks of gestation, with the highest incidence (13%) among infants weighing <1,000 g. Furthermore, overall survival has not changed in the past five decades and the average mortality from NEC is 20–30%, with mortality as high as 50% in those infants requiring surgical management.²⁵ Though the majority of cases of NEC are found among premature infants, a small section of term neonates or shortly before (that is, 35 weeks of gestation) develop NEC, frequently in association with other conditions.²²

Comparison between birth weight, risk of NEC and mortality²⁷

Birth weight(grams)	Risk of NEC (%)	Mortality with NEC (%)
501-750	12	42
751-1000	9	29
1001-1250	6	21
1251-1500	3	16

Gestational Age and risk of developing NEC²⁷

Gestational Age (weeks)	Risk of NEC (%)
22	11
23	16
24	11
25	9
26	10
27	8
28	8

India

The incidence of NEC in preterm neonates < 32 weeks of gestation was 5.2%. Mortality rates vary across centres and range from 10 to 40% depending on gestational age of the baby.^{28,29}

Risk factors and etiology^{27,30-32}

Factors related to the infant

- Prematurity (highest risk with lowest gestational age)
- Very low birth weight (<1,500 g)
- Low Apgar score at 5 min
- Formula feeding
- Mechanical ventilation
- Congenital defects
 - Congenital heart disease
 - Patent ductus arteriosus
 - Gastroschisis
- Pharmacological interventions
 - Indomethacin
 - Histamine H2 receptor antagonists
 - Prolonged empirical antibiotic use (5 days)
 - Concomitant use of indomethacin and glucocorticoids
 - Indomethacin tocolysis

- Anaemia

Factors related to the mother

- Human immunodeficiency virus HIV-positive status
- Illicit drug abuse (including opiates, cannabinoids and cocaine)
- Chorioamnionitis
- Vaginal delivery

Factors Making Premature Infant's Gut Susceptible to NEC

Mechanical factors (barrier integrity)

- Decreased peristalsis,
- Mucus layer deficiency,
- Composition of lipids (premature gut is more permeable).

Bacterial factors:

- Delayed or altered bacterial colonization,
- Paucity of anaerobic bacteria.

Miscellaneous:

- Decreased gastric acid production
- Decreased lactase levels,
- Decreased bile acids (insufficient to form bile micelles).

Risk factors for necrotizing enterocolitis by infant category³³

Premature infants (<32 weeks)	All gestational Ages	Late preterm and term infants
Very low birthweight (<1500g)	Formula feeding	Congenital heart disease
Small for gestational age	Hypoxia	Chromosomal abnormalities
Anemia in need of packed red blood cell transfusion	Hypotension requiring inotropic support	Gastroschisis
Patent ductus arteriosus	Birth asphyxia	Sepsis
	Intrauterine growth restriction	Postnatal respiratory distress
	Polycythemia	Hypoxic ischemic encephalopathy
	Chorioamnionitis	Milk protein allergy
	Exchange transfusion	Hypothyroidism
	Umbilical lines	Protracted diarrhea
	Premature rupture of membranes	Maternal history of preeclampsia
	Maternal cocaine use	Maternal history of gestational diabetes
	Severe anemia	
	Idiopathic	

Pathology

NEC predominantly affects the ileum and colon, the commonest location being ileocecal area. It can affect whole of gastrointestinal tract in severe cases. On gross examination, distended bowel loops are seen with areas of congestion, hemorrhage, necrosis, and pneumatosis.



Figure 1. Necrotic bowel loops of Necrotizing enterocolitis on Macroscopic appearance²⁷

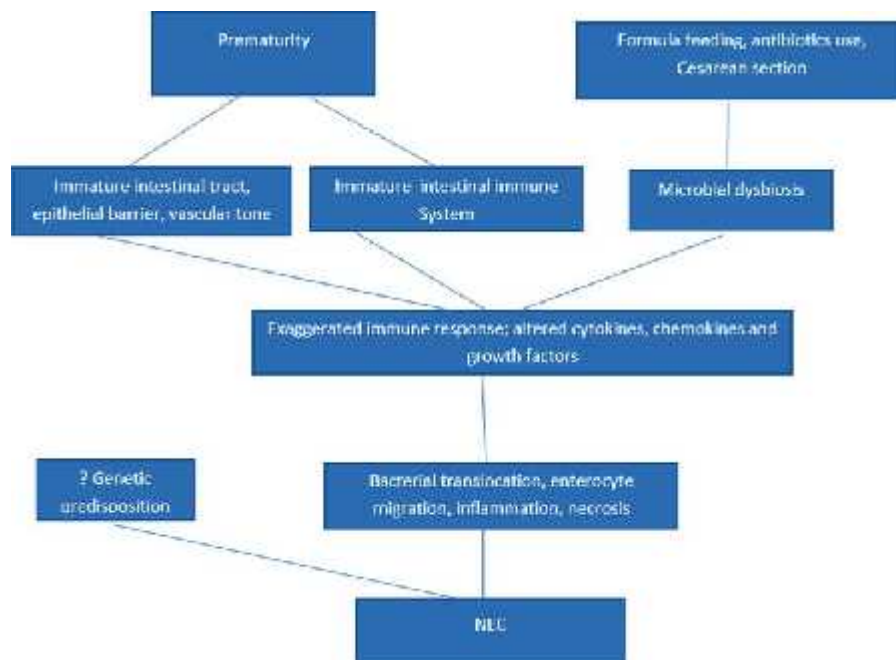


Figure 2. Schematic presentation of pathogenesis of NEC²⁷

Microscopically, the predominant feature is coagulation necrosis, suggesting an ischemic origin of NEC. The aggregated inflammatory cells are both acute and chronic, such as neutrophils, lymphocytes, and macrophages representing an appropriate response to pathogenic bacterial invasion and tissue necrosis. Stage of reparative histological process is marked by granulation tissue formation, epithelial regeneration and fibrosis. Common pathogens isolated in NEC are Enterobacteriaceae including *Enterobacter*, *Escherichia*, *Salmonella*, and *Klebsiella* (68%); Staphylococcal species (26%); Clostridium species (4%); viruses including rota, echo, corona, and toro (11%); and candida (1%).²⁷

Pathophysiology relative to infant risk factors

Is multifactorial and complex. It has been proposed by some researchers that the definition of NEC be individualized to include risk factors associated with the onset of disease. Three general features underlie NEC in all infants: 1) dysbiosis, 2) injury to the intestinal lining, and 3) activation of an immune response. Dysbiosis is the abnormal balance of gut microbiota favouring opportunistic and pathogenic bacteria, or pathobionts. The other underlying features include injury to the intestinal lining and activation of proinflammatory immune responses in the gut and periphery.³³

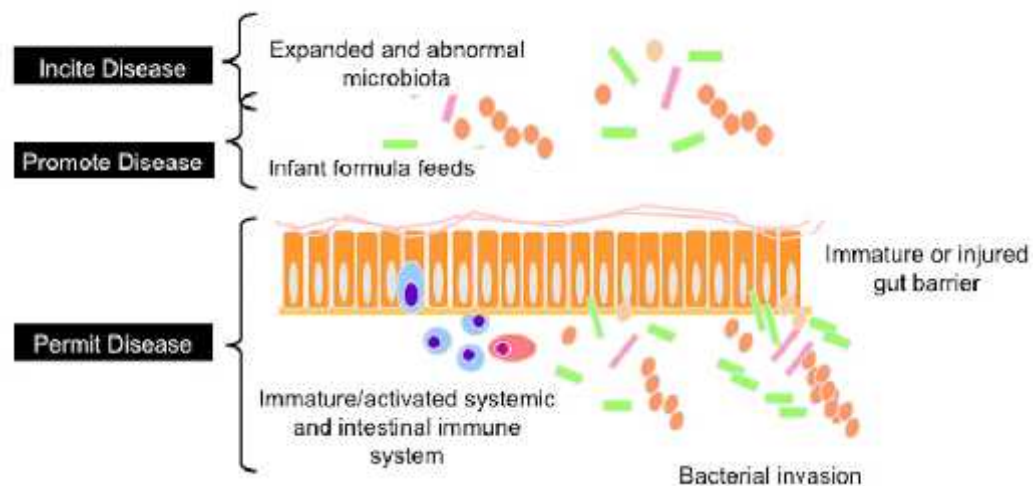


Figure 3. The pathogenic sequence of (NEC) with the factors that may incite, promote, and permit the pathogenic sequence of NEC.³³

Factors that incite NEC may do so through injury to the intestinal barrier resulting from decreased bowel perfusion or after direct damage to the intestine from bacterial metabolites, toxins or infant formula feeds. Poorly digested formula, antibiotics may then result in dysbiosis or bacterial overgrowth conditions that promote disease. Finally, circumstances unique to each infant, such as prematurity, congenital heart disease, or sepsis, are factors that permit NEC to occur. These factors, taken together, result in the conditions necessary for NEC, including bacterial overgrowth, loss of bowel wall integrity, and the generation of proinflammatory immune responses triggering bacterial invasion in the gut and disease progression. Next-generation sequencing of stool demonstrated dysbiosis before the evolution of NEC. Pathobionts present in the microbiota of the gut in infants who develop NEC may, therefore, be "bystanders" rather than the primary cause of disease. The inflammatory cascade generated in response to bacterial overgrowth may additionally damage the epithelium, leading to increased intestinal permeability and translocation

of bacteria. Furthermore, immune activation and often a systemic inflammatory response syndrome results from decreased barrier function, leading to proinflammatory cytokine-mediated injury in the brain and subsequent neurodevelopmental injury.³³

The development of NEC after red blood cell transfusion, so-called transfusion-associated NEC (TNEC), has been recognized for 2 decades, although the mechanism remains poorly understood. TNEC developed in infants born at earlier gestational ages with a history of packed Red Blood Cells (PRBC) transfusion. In addition, TNEC developed at later postnatal ages than did gestational age-matched controls, with an average onset at 3 to 5 weeks of postnatal age compared with 1 to 3 weeks of postnatal age in VLBW infants with NEC unrelated to transfusion. One controversial risk factor for TNEC may result from increased iron handling in the intestinal epithelium in response to the recycling of red blood cells after transfusion. Other authors suggest that PRBC transfusion may not cause NEC directly but that symptomatic anemia is an independent risk for NEC. Example, infants with transfusion-related acute gut injury had a larger degree of anemia with lower hematocrit levels. It is, therefore, possible that anemia results in decreased oxygen delivery to the gut, rendering the mucosa at increased risk for injury associated with feeding, a state that is suddenly reversed with PRBC transfusion, resulting in a reperfusion-type mucosal gut injury.³³

Clinical presentation and diagnosis

The clinical symptoms in infants later confirmed to have NEC may vary from subtle to fulminant. Infants presenting with fulminant disease may have either focal abdominal signs and symptoms or nonspecific systemic signs and symptoms with cardio respiratory collapse indistinguishable from sepsis, metabolic disease, or critical congenital heart disease. The diagnosis of NEC is suspected from infant demographics and clinical presentation, but is confirmed by the presence of pneumatosis intestinalis on abdominal radiographs.³³

Systemic signs and symptoms may include lethargy, temperature instability, new or worsening apnea, bradycardia, decreased perfusion, mottling, and hypotension.³³

Abnormal laboratory tests often encountered in patients with NEC, although not specific to NEC alone, are the presence of thrombocytopenia, neutropenia, elevated C-reactive protein level, metabolic acidosis, electrolyte abnormalities, and coagulopathy. Similar to the onset of NEC, disease progression may follow a slow and stepwise deterioration, or it may be rapid in onset, with fulminant progression and death.³³

The Bell system¹⁷⁻¹⁹ was proposed nearly 40 years ago to enable uniform clinical staging stratifying infants into the categories of suspected (stage I), definite (stage II), and advanced (stage III) NEC based on clinical and radiographic findings.

Modified Bell staging in NEC³⁵

Bell stage	Clinical	Radiographic	Gastrointestinal
I	Apnea and bradycardia, temperature instability	Normal gas pattern or mild ileus	Gastric residuals, occult blood in stool, mild abdominal distention
IIA	Apnea and bradycardia, temperature instability	Ileus gas pattern with 1 dilated loops and focal pneumatosis	Grossly bloody stools, prominent abdominal distention, absent bowel sounds
IIB	Thrombocytopenia and mild metabolic acidosis	Widespread pneumatosis, ascites, portal venous gas	Abdominal wall edema with palpable loops and tenderness
IIIA	Mixed acidosis, oliguria, hypotension, coagulopathy	Prominent bowel loops, worsening ascites, no free air	Worsening wall edema, erythema and induration
IIIB	Shock, deterioration in laboratory values and vital signs	Pneumoperitoneum	Perforated bowel

A subsequent version, modified Bell staging, included additional clinical signs and symptoms and predicted the likelihood of surgery. The signs and symptoms of suspected or stage I NEC overlap with feeding intolerance, whereas stage II NEC is defined by the presence of pneumatosis intestinalis on abdominal radiographs.^{33,35}

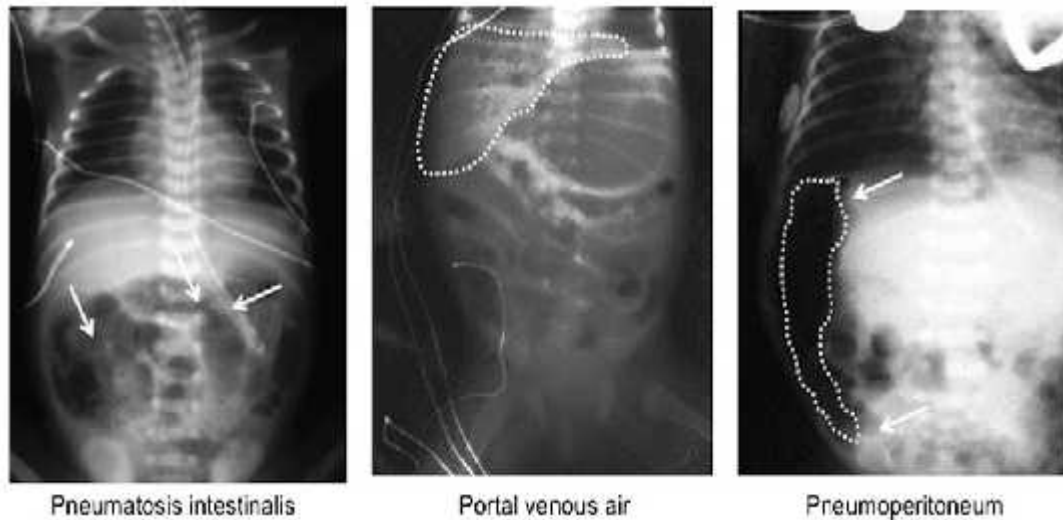


Figure 4. Radiographic appearance of necrotizing enterocolitis. Abdominal radiographic findings include pneumatosis intestinalis, visible as linear streaks (arrows) and "bubbly" appearance throughout the bowel; portal venous air noted throughout the liver (inside white dotted line) and dilated and stacked loops of bowel with thickened walls; and pneumoperitoneum visible as an air pocket (encircled by white dotted line and arrows) overlying the liver in the righted left lateral decubitus view.³³

Pneumatosis intestinalis is pathognomonic for NEC and results from intramural gas generated during anaerobic bacterial metabolism that becomes trapped in the submucosal layer of the bowel wall.³³

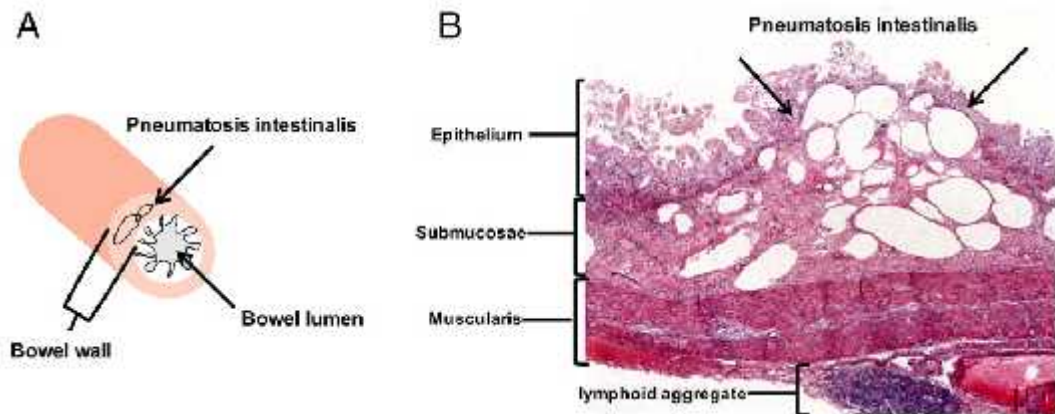


Figure 5 A.Schematic representation of the small bowel demonstrating the location of pneumatosis intestinalis in the bowel wall(arrows).

Figure 5 B.Histologic features of necrotizing enterocolitis on a light micrograph of a segment of the small bowel stained with hematoxylin and eosin. Visible are destruction of the intestinal epithelium and villi ,pneumatosis intestinalis in the submucosae and inflammatory cells distributed throughout the bowel wall, and a lymphoid aggregate/lymph node in the serosal layer.³³

Stage II NEC is a surgical emergency if injury progresses to full thickness destruction of the bowel wall, leading to intestinal perforation. Intestinal perforation is visible as portal venous and free intraperitoneal air on abdominal radiograph. Progression to stage III NEC often leads to hemodynamic instability and respiratory compromise requiring cardiorespiratory life support. Bell staging has also been shown to be useful in predicting disease outcomes as well as the likelihood of surgical intervention in NEC. For example, infants with stage II NEC are at higher risk for perforation and surgical intervention, correlating with increased morbidity and mortality.³³

NEC monitoring by clinical, laboratory, and radiographic parameters is crucial. Serial abdominal radiography is considered the gold standard to evaluate infants for disease progression. Radiography is used to determine the presence of pneumatosis intestinalis, pneumoperitoneum (free air in the abdominal cavity), or portal venous air. Intestinal perforation, as indicated by the presence of pneumoperitoneum, requires immediate surgical evaluation and intervention. The radiographic appearance of abdominal free air may, however, be subtle and may become apparent in only 60% of infants with identified perforation intraoperatively. Abdominal ultrasonography may be used as an adjunct to radiography in diagnosing NEC, with positive findings of pneumoperitoneum, focal fluid collection, portal venous gas, pneumatosis intestinalis, and Doppler-identified areas of bowel hypoperfusion. However, expertise in abdominal sonography may not be widely available and is limited by observer variability. Biomarkers in stool, urine, and serum to identify or confirm infants with early-stage or progressive NEC are under evaluation. Thus, having a high index of suspicion, performing serial physical examinations, and closely following the patient's laboratory studies and abdominal radiographs, remain the standard for diagnosing NEC.³³

Factors related to prematurity that increase the susceptibility to NEC⁵

- Increased expression of the innate immune receptor Toll-like receptor 4 (TLR4) in the apical surface of enterocytes and within intestinal stem cells.
- Increased baseline levels of endoplasmic reticulum (ER) stress within intestinal crypts.
- Decreased number of mucus-producing goblet cells.
- Impaired intestinal motility.
- Decreased digestion and absorption

- Enterocyte immaturity
- Impaired regulation of microcirculatory perfusion of the gut
- Increased bile acid levels and decreased bile acid-binding protein in the intestinal lumen
- Tight junction immaturity and impairment
- Inefficient antigen processing and presentation
- Impaired intestinal regeneration and healing
- Discontinuation of gut exposure to amniotic fluid
- Increased levels of platelet-activating factor (increased production and decreased degradation) and increased expression of its receptor in the ileum
- Decreased forkhead/winged helix transcription factor⁺ regulatory T cell levels in the small intestine
- Decreased levels of intraepithelial lymphocytes expressing the T cell receptor
- Decreased intestinal expression of transforming growth factor 2.⁵

Differentiating medical and surgical NEC: use of biomarkers

Biochemical markers which are widely used for bowel necrosis are platelet count (99%), C-reactive protein (CRP) (90%), white blood cell count (83%), lactate levels (43%), fecal calprotectin (10%), and interleukin (IL)-6 or interleukin-8(10%).³⁶ Local Bell stage II and systemic Bell stage III NEC can be differentiated by Fecal calprotectin which is a marker of intestinal inflammation with 76% sensitivity and 92% specificity. Fecal levels of another protein, S100A12, are noted to be higher in infants with suspected NEC who subsequently develop bowel perforation. Unremitting and relentlessly high CRP levels despite treatment may indicate advanced stage of NEC and bowel necrosis. It is noted that IL-8 levels is significantly

elevated in patients developing surgical NEC. The levels can discriminate NEC totalis from focal and multifocal diseases and also predicts 60-day mortality. Maximum concentration of CRP and duration of CRP elevation are increased in infants who developed intestinal strictures following NEC, while the negative predictive value of CRP levels <10 mg/dL for stricture development is 100%.²⁷

Intestinal fatty acid-binding protein (I-FABP), which is found in mature enterocytes is released after cell disruption into blood stream and is later excreted in the urine, is a marker of intestinal injury and severe NEC.³⁷

Other biomarkers being investigated for surgical NEC are serum amyloid A protein, urine peptides, liver fatty acid-binding protein, and heart rate characteristic index.²⁷

Management principles of NEC

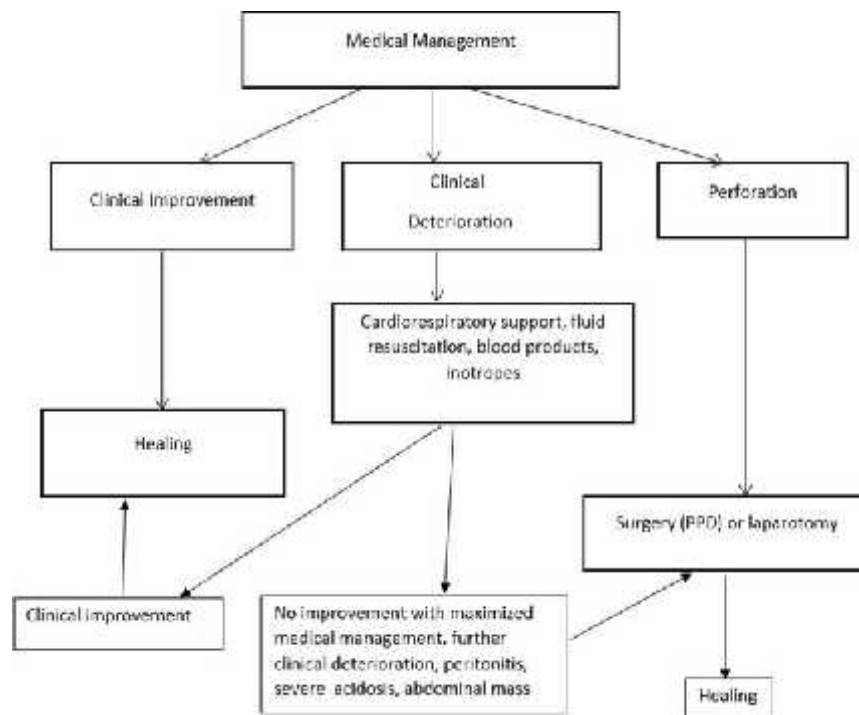


Figure 6. Flow chart outlining management principles in NEC.²⁷

NEC is suspected on clinical presentation: which can include feeding intolerance, abdominal distention, bloody stools, emesis, and gastric retention. These clinical signs when present require further investigations which include blood tests to detect probable thrombocytopenia or metabolic acidosis and radiographic imaging that detects distended bowel loops, pneumatosis intestinalis, and portal venous gas. Depending upon imaging results and clinical signs, surgical management is taken into consideration. X-ray abdomen and ultrasonography have proved to be effective modalities to monitor the progression of NEC.³⁸

1. For Bell stage I (suspected NEC), only supportive medical care is provided.
2. For Bell stage II (proven NEC), management includes antibiotic treatment, nasogastric decompression, and total parenteral nutrition. Failure to respond subsequently leads to indication of surgical management.³⁹
3. Advanced cases of NEC can be treated medically sometimes necessitating the use of inotropes. Nonetheless neonates with intestinal perforation or suspected bowel necrosis or those who do not respond to medical management require surgical management.
4. It is noted that 27-52% of neonates weighing <1000 gms requires surgical intervention.⁴⁰

Outcomes of infants with NEC

Morbidity in children with NEC is as high as 20-50% with increased incidence of recurrence, intestinal strictures, short bowel syndrome, growth delay and neurodevelopmental impairment. Longer hospitalization stays, increased mortality rate and higher financial costs compared with preterm neonates without NEC have been noted.⁵

Long term survivors of NEC are frequently affected by neurodevelopmental impairment, demonstrated by their impaired cognitive and developmental assessments highlighting the extensive sequelae of this disease.⁵

Complications and outcomes in patients with NEC⁵

Type of complication or outcome	Incidence	Associated factors
Recurrence	4–10%	<ul style="list-style-type: none"> • Nonoperative management, congenital heart disease
Mortality	15–63%	<ul style="list-style-type: none"> • Main predictor is gestational age • Patients managed surgically have the highest mortality
Intestinal strictures	12–35%	<ul style="list-style-type: none"> • Most frequent in patients managed medically • Affects colon in up to 80%
Stoma complications	50%	<ul style="list-style-type: none"> • Most common include: prolapse, stricture and retraction • Proximal jejunostomies can cause substantial electrolyte and fluid losses, impaired weight gain and peristomal skin complications
Short Bowel Syndrome	20–35%	<ul style="list-style-type: none"> • Relative risk up to 85.9 (95% CI 45.8–160.9) • Increased risk associated with a residual intestinal length <25% of predicted for gestational age
Neurodevelopmental impairment	30–50%	NEC vs. no NEC (OR: 1.82). Surgical NEC versus medical NEC (OR: 2.34)
Growth delay	10%	<ul style="list-style-type: none"> • Affected children fall below 50th percentile for weight and height • Problem more severe in patients with short bowel syndrome after NEC compared with age-matched controls without NEC

Preventative treatments

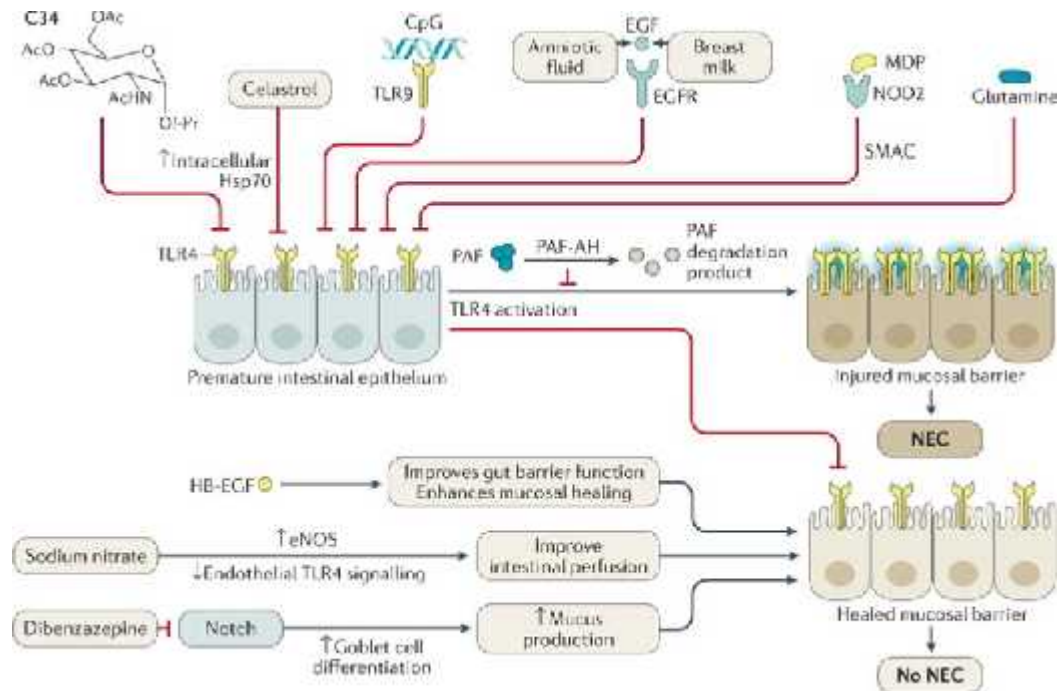


Figure 7. Factors that attenuate or prevent the development of NEC in experimental models⁵

Activation of Toll-like receptor 4 (TLR4) plays an essential part in occurrence of necrotizing enterocolitis (NEC) by increasing enterocyte and intestinal stem cell apoptosis and impairing mucosal healing through decreased restitution and proliferation. These events lead to disruption of the epithelial barrier, which allows luminal bacteria to translocate and trigger a systemic inflammatory response, multiple organ failure and death.⁵

Exogenous factors include: the small-molecule TLR4 inhibitor C34 (bacterial (CpG) DNA, muramyl dipeptide (MDP), celastrol (also known as tripterine) sodium nitrate, endothelial nitric oxide synthase, Heat shock protein 70 (Hsp70) and dibenzazepine.⁵

Probiotics

In neonates weighing <1,500 grams, the use of prophylactic probiotics reduced the incidence of NEC (RR 0.34, 95% CI of 0.23–0.50), the risk of NEC-associated mortality (RR 0.56, 95% CI of 0.34–0.93), and the total length of hospital stay. Notably, however, probiotic administration did not significantly reduce the risk of developing NEC in infants with a birth weight of <1,000 grams or the risk of developing NEC requiring surgery in infants of any size (RR 0.56, 95% CI of 0.56–1.25).^{5,41-43}

The prophylactic use of probiotics in preterm neonates with a birth weight of <2,500 grams to reduce the risk of NEC is suggested by American Pediatric Surgical Association (APSA) based on clinical trials and Cochrane reviews.^{5,41-43}

Antenatal glucocorticoids

Various randomized control trials have reported that the use of antenatal glucocorticoids has reduced the incidence of NEC by reducing bacterial translocation, decreasing colonization with aerobic bacteria and by increasing the activity of enzymes such as sucrase, maltase, lactase and Na/K-ATPase.⁴⁴⁻⁴⁸

Restrict Empiric antibiotic use:

A meta-analysis comprising of 9 studies (randomized control trials and retrospective cohort studies) on 5207 preterm and LBW neonates concluded that the use of prophylactic antibiotics for high - risk preterm neonates has no role in decreasing the incidence of NEC.⁴⁴⁻⁴⁸

Fluid restriction:

Meta-analysis have shown that restriction of fluid intake, without allowing significant dehydration, perhaps decreases the risk of death from NEC without any adversities.⁴⁹⁻⁵⁰

Erythropoietin (EPO)

EPO is a protecting constituent of amniotic fluid and breast milk which helps in intestinal development ,and intestinal restitution , current retrospective cohort study done by Ledbetter have shown that recombinant human erythropoietin (rEPO) inhibits NO formation thus preventing NEC as those neonates who received rEPO has incidence of NEC of 4.6% vs 10.8% in neonates who did not received rEPO.^{51,52}

Nutritional approaches for NEC prevention: the use of breast milk

Various systemic reviews and randomized control trials have shown that breast milk statistically significantly decreases the incidence of NEC. Human milk contains a variety of beneficial bioactive factors, breast milk derived exosomes and human milk oligosaccharides (HMOs) among which several have been shown to reduce NEC incidence and progression.⁵³

NEC-protective factors in human milk

- Nitrate and/or nitrite and antioxidant factors
- L-arginine
- Human milk oligosaccharides and prebiotics
- Lactoferrin
- Secretory IgA

- Platelet-activating factor acetylhydrolase
- Growth factors:
 - Epidermal growth factor
 - Heparin-binding epidermal growth factor-like growth factor
 - Transforming growth factor 2
 - Erythropoietin

Breastfeeding is the gold standard method of feeding because human milk is the best milk which is tailor-made and uniquely suited to the human infant. Sushruta, an ancient Indian surgeon, has beautifully described mother's milk in his Samhita, "One just cannot compare even water of seven seas with mother's milk, which is nothing but water ensuring optimum growth, nutrition, and healthy life of hundred years." All mothers should be encouraged to breast-feed their infants. When a mother, for some reason, is unable to feed her infant directly, her breastmilk should be expressed and fed to the infant .It is universally accepted that human milk is the optimum source of nutrition for the first six months of life.⁵⁴

Various observational studies and RCTs have shown that human milk is protective against both NEC and sepsis compared to formula feeds as it has immune modulating and bioactive substances which are bactericidal.⁵⁴

Shoji et al.⁵⁵ In their study has supported the theory that breastmilk has antioxidant properties with a protective effect from oxidative stress.

Many infants lack access to their mother's own milk (MOM) because of issues related to the mother's postpartum death or illness, abandonment, latchment difficulties, or delay in milk production. Under these circumstances when mothers

own milk (MOM) is not available WHO recommends pasteurized donor human as the next best alternative especially for LBW babies⁸.

Pasteurized donor human milk (PDHM) is distributed through Human milk bank which systematically collects; pasteurizes, stores and distribute the milk, PDHM is generally pasteurized to prevent the potential risk for the transmission of pathogens from donor mothers to preterm infants. Its safety must be considered accurately because pasteurization reduces the concentration of immunological proteins in human milk.⁵⁶

Modern human milk banking is in its infancy in India. Lack of awareness, leadership deficit, infrastructural and maintenance costs, and fewer neonatal setups are some reasons for the same. Asia's first milk bank with the name of Sneha, founded by Dr. Armeda Fernandez, was started in Dharavi, Mumbai on November 27, 1989. As of now there are nearly 50 milk banks all over India which is still much less as compared to the growth of NICUs. The promotion of formula milk by the industries overlays the awareness about the human milk banks. There is need to establish more human milk banks in level II and level III NICU facilities as it protects the preterm neonates from the problems arising from the use of formula feeds.⁵⁷

There are meta-analysis and systemic reviews among PDHM and non-breast milk feeds which have shown lower incidence of NEC, may be due to supply of immunoprotective factors to the immature mucosa.^{56,58-60}

Narayan et al.⁶¹ showed reduction in infection being 10.5% with PDHM which was much lesser compared to 33.3% with formula feeds.⁶¹

There are multiple systemic reviews and RCTs which has shown decreased incidence of NEC and sepsis with PDHM as compared to formula feeds.⁶²⁻⁶⁵

In a systemic review done by Boyd et al,⁵⁹ 2007, comprising of 3 studies including 268 infants compared diet of unfortified donor human milk (DHM) vs formula results shown that DHM was associated with a decreased incidence of NEC (RR 0.21; 95% CI 0.06-0.76).

Sisk PM et al.⁶⁶ in 2007 reported a prospective cohort study to ascertain whether (50% or more) human milk (HM) as enteral feeding in the initial 14 days of life are defensive against NEC . 202 neonates were enrolled. Confirmed NEC was noted in 5/46 (10.6%) neonates belonging to low human milk (LHM) group vs 5/156 (3.2%) neonates of high human milk (HHM). Gestational age was the only perinatal factor associated with risk of NEC. After adjustment for gestational age, HHM was associated with a lower risk of NEC (OR=0.17, 95% CI: 0.04 to 0.68), P=0.01). It concluded that, enteral feeding was associated with six-fold decrease in the incidence of NEC when enteral feeding given in first 14 days of life contained atleast 50% of HM.

A meta-analysis done by Quigley and McGuire⁶⁷ in 2014 of 6 RCTs including 869 infants comparing DHM vs formula shown that DHM was associated with low incidence of NEC (RR.2.77; 95% CI, 1.40-5.46).⁶⁷

Kantorowska et al.⁶⁸ In 2016 did a retrospective study including 10823 infants weighing <1500 gm from 22 hospitals and compared DHM vs formula it was noted that DHM associated with a decrease incidence of NEC from 5.7 % to 2.9 % (P = .0006).

Chowning et al.⁶⁹ reported a retrospective cohort study in 2015 with 550 neonates of gestation being <35 wk and weighing <1500gm shown that DHM was associated with a decreased incidence of NEC (13.5% vs 3.4% ; P<.001).

Schanler et al.¹³ described that volumes of at least 50ml/kg/day of HM lowered the NEC incidence, it was found that those neonates who were healthier would have got rapid increment of feeding volume and thus being confounding the results.

Altobelli E et al.⁶³ reported a systematic review and meta-analysis in 2020 and found that, there was risk reduction of NEC with human milk vs formula: (Relative risk [RR]=0.62 (0.42–0.93).

Figure 8 Impact of Donor milk⁷²

Sepsis	<ul style="list-style-type: none"> • Donor human milk reduced the risk of late-onset sepsis in vulnerable, low-birthweight infants by 19% in the first 28 days • Donor human milk has a greater protective effect compared with formula.
Necrotizing enterocolitis	<ul style="list-style-type: none"> • Human milk feeding, whether mother’s own milk or donor human milk reduces necrotizing enterocolitis by as much as 79% when formula is avoided. • Four systematic reviews across study designs and countries found that donor human milk protects preterm infants against necrotizing enterocolitis more than formula.
Retinopathy of prematurity	<ul style="list-style-type: none"> • Human milk feeding in the NICU is associated with lower rates of severe retinopathy of prematurity.
Feeding tolerance	<ul style="list-style-type: none"> • Preterm infants fed unfortified donor human milk had greater feeding tolerance, fewer vomits, less gastric stasis, and reduced diarrhea compared with formula-fed infants.
Reduced length of stay in NICU	<ul style="list-style-type: none"> • Cost of providing donor human milk to preterm infants is mitigated by a reduced risk of complications and shorter length of stay in NICU. • Fewer hospital readmissions for illness in the year after NICU discharge have been noted.
Cost saving	<ul style="list-style-type: none"> • The percentage of infants who are exclusively breastfed at discharge is about 13% higher in NICUs with a human milk bank. • Saving US \$ 8,167 per infant using donor human milk through shortened length of stay and reduced cases of necrotizing enterocolitis and sepsis. • Estimated savings to NICU or health care plan for every dollar spent on donor human milk: US\$11. • In Brazil, the national human milk banking network saves \$ 540 million in health care costs annually.
Neurodevelopmental outcomes and long-term benefits	<ul style="list-style-type: none"> • Later in childhood and adulthood, preterm infants fed human milk have been shown to have lower rates of metabolic syndrome, increased white matter and brain volume, and significantly greater scores for mental, motor, and behavior ratings.

There are limited data regarding PDHM and NEC in Indian context. In view of this; this cross - sectional study was planned to find out the incidence of NEC among preterm neonates receiving PDHM; to assess acceptability of PDHM in recipients and to evaluate the outcomes among preterm neonates receiving PDHM with respect to sepsis, mortality rate, duration of NICU stay, growth parameters (weight, length, head circumference) and time of initiation of breastfeeding.

METHODOLOGY

This study was conducted from January 2019 to February 2020 in the Department of Pediatrics, KLES Dr. Prabhakar Kore Hospital and Medical Research Centre, Belagavi.

Study design

Hospital based cross-sectional study.

Study duration and period

January 2019 to february 2020.

Place

This study was conducted in the Department of Pediatrics, KLES Dr. Prabhakar Kore Hospital and Medical Research Centre, Belagavi a tertiary care teaching hospital attached to , KAHER Jawaharlal Nehru Medical College , Belagavi.

Source of data

Preterm neonates admitted in the NICU of, KLES Dr. Prabhakar Kore Hospital and Medical Research Centre, Belagavi

Sample size and calculation

The sample size was calculated from the formula as mentioned below.

$$n=1.96^2 * p q / d^2$$

Where,

n = Sample size

p = Prevalence of the preterm birth which was 16% (babies born < 37 weeks).¹

Q = 100-p (when p is in percentage)

d = Precision of the estimate 5% at 95% CI

Therefore,

$$n = 1.96^2 \times 16 \times 84 / 5^2$$

$$n = 3.84 \times 16 \times 84 / 25$$

$$n = 5160.96 / 25$$

$$n = 206.43 \quad 207$$

Calculated sample size was 207 and actual sample size was 221, preterm neonates admitted to NICU fulfilling the selection criteria were enrolled.

Selection Criteria

Inclusion Criteria

- Preterm admitted to NICU on feeds.
- Preterm neonates of mothers with postpartum illness
- Preterm neonates whose mothers had temporary lactation failure
- Abandoned preterm neonates.
- Preterm neonates whose mothers died in immediate postpartum period.

Exclusion criteria

- Preterm neonates with major congenital anomalies
- Surgical problems of the gastrointestinal tract
- Birth asphyxia
- Early onset sepsis.

Method of collection of data

The study was conducted after the approval from the ethical committee of the institution ,the parents of neonates fulfilling selection criteria were briefed about the nature of the study and a written informed consent was obtained from parents/caregivers to participate in the study prior to the enrollment (Annexure II). Parents/caregivers of the neonates who fulfilled the selection criteria were interviewed and demographic data including age, gender and educational status of the parents, family members, family income and socio-economic status according to the Modified B. G. Prasad's Classification⁷³ were noted in a pretested questionnaire.

At admission baseline parameters were recorded which were maternal history, maternal age, gravida, antenatal care, antenatal risk factors, birth history of the neonates like, mode of delivery, gestational age, modified Ballard score, APGAR score and indication for NICU admission. The neonates were subjected to general physical examination followed by systemic examination and all these findings were recorded on a pre-designed and pre-tested proforma. The neonates were followed up for feeding type, method, development of NEC, sepsis, growth parameters till discharge or 28 days whichever was earlier. PDHM was given from human milk bank of KAHER Dr. Prabhakar Kore Charitable Hospital, Belagavi to the neonates.

OUTCOMES ASSESSED:

Incidence of NEC: “Incidence refers to the number of NEW cases occurring in a defined population during a specified period of time”.⁷⁴

“Necrotizing enterocolitis (NEC) is an ischemic and inflammatory necrosis of the bowel primarily affecting premature neonates after the initiation of enteral feeding”.⁷⁵

Criteria for diagnosis

Modified Bell staging in NEC³⁵

Bell stage	Clinical	Radiographic	Gastrointestinal
I	Apnea and bradycardia, temperature instability	Normal gas pattern or mild ileus	Gastric residuals, occult blood in stool, mild abdominal distention
IIA	Apnea and bradycardia, temperature instability	Ileus gas pattern with 1 dilated loops and focal pneumatosis	Grossly bloody stools, prominent abdominal distention, absent bowel sounds
IIB	Thrombocytopenia and mild metabolic acidosis	Widespread pneumatosis, ascites, portal venous gas	Abdominal wall edema with palpable loops and tenderness
IIIA	Mixed acidosis, oliguria, hypotension, coagulopathy	Prominent bowel loops, worsening ascites, no free air	Worsening wall edema, erythema and induration
IIIB	Shock, deterioration in laboratory values and vital signs	Pneumoperitoneum	Perforated bowel

Stage II and more will be considered as confirmed NEC³⁵

Secondary outcomes

Acceptability of PDHM: The parents / caregivers of the neonates fulfilling the selection criteria were briefed about the nature of the study and depending upon their consent acceptability of PDHM was assessed.

Feeding details:

Pasteurized donor human milk (PDHM): “Breast milk expressed by a mother that is then processed by a donor milk bank for use by a recipient that is not the mothers own baby”.⁷⁶

Formula feeds: “An artificial substitute for breast milk intended for feeding infants, using cow’s milk as a base, supplemented with vitamins and minerals”.⁷⁷

Method - Nasogastric feeding: “A feeding tube is a small, soft, plastic tube placed through the nose (NG) or mouth (OG) into stomach to provide feeds and medicines to the babies after measuring the distance from either the nostril or the mouth (depending on insertion site) to the tragus (lobe of the ear) to the half way point between the xiphisternum and the umblicus”.⁷⁸

Spoon / Paladai feeding: “The paladai is a cup-like utensil with a narrow tip has been used traditionally to feed preterm neonates who has not developed coordinated suck-swallow reflex”.⁷⁹

Sepsis: “Neonatal sepsis is a clinical syndrome of systemic illness accompanied by bacteremia occurring in the first month of life” which will be labelled after proven blood culture.⁸⁰

Mortality: “Neonatal mortality - Number of neonatal deaths in a given year per 1000 live births in that year”⁷⁵

Anthropometric measurements:⁸¹

Weight: The neonate weight was measured at admission on Seca 334 weighing scale with precision of 0.001 kg, neonate was weighed naked with no clothing or diaper after making sure that scale was placed on flat, hard, even surface. Three readings were noted and mean was taken.

Length: The neonate length was measured at admission on Seca 417 baby length board after placing it on a horizontal and level surface. Three measurements for each baby was taken and mean was taken after measuring it to nearest 0.1 cm.

Head circumference: Neonates head circumference was measured with Schorr tape with precision of 1 mm by placing it over the occipital protuberance at the back and just over the supraorbital ridge and the glabella in front, once being positioned correctly it was pull tight to compress the hair and the skin, but not too tight causing injury to the baby. Three measurements were taken for each baby and mean was taken of it.

Statistical analysis

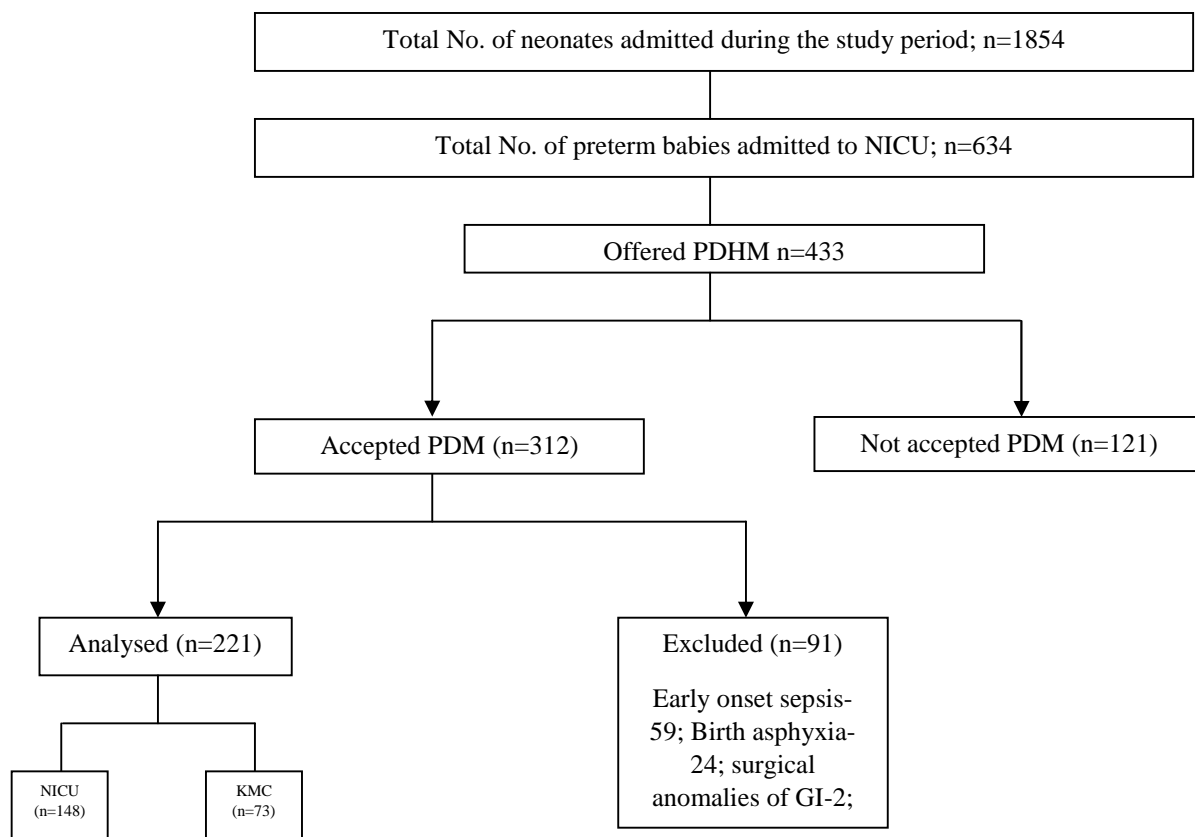
The data obtained was coded and entered into Microsoft Excel spreadsheet. Data was analysed using IBM SPSS Statistics 20 Windows (SPSS Inc., Chicago, US). The categorical data was expressed in terms of rates, ratios and percentages and the comparison was done by either chi square test or Fisher's exact test. The incidence of NEC and acceptance of PDHM was expressed in terms of percentages. The association between NEC and various demographic parameters was tested using chi square test or fishers exact test. For all the continuous variables, the results were either given in Mean \pm SD and/or median and interquartile range (IQR). The normality of the data was assessed by Shapiro Wilk test. The comparison of continuous variables was tested using paired t test. A probability value (p value) of less than or equal to 0.050 at 95% confidence interval was considered as statistically significant.

RESULTS

The cross-sectional study was conducted from January 2019 to February 2020 in the Department of Pediatrics, KLES Dr. PrabhakarKore Hospital and Medical Research Centre, Belagavi.

A total of 1854 neonates were admitted in NICU during the study period of which 634 were preterms. Of these, 433 preterm neonates were offered PDHM as other 201 were on breastfeed. Of these, 433 preterms; 312 accepted PDHM which were enrolled in study of which 91 were excluded and 221 were analysed. The selection enrolment of the neonates is as shown in CONSORT Diagram below.

Figure 9. CONSORT diagram for screening and enrolment of newborns



I. MATERNAL SOCIODEMOGRAPHIC PROFILE

Table 1. Distribution of preterm neonates according to the maternal and sociodemographic profile

Maternal and sociodemographic characteristics	Findings	Distribution (n=221)	
		Number	Percentage
Age group	18 to 20	93	42.08
	21 to 25	82	37.10
	26 to 30	27	12.21
	31 to 35	12	5.42
	>35	7	3.16
Educational status	Illiterate	23	10.41
	Primary	101	45.70
	Secondary	79	35.75
	Graduate	18	8.14
Religion	Hindu	184	83.26
	Muslim	36	16.29
	Others	1	0.45
Occupation	Home maker	209	94.57
	Employed	11	4.98
	Self employed	1	0.45
Socio economic status	Class I	12	5.43
	Class II	40	18.10
	Class III	74	33.48
	Class IV	89	40.27
	Class V	6	2.71

In the present study, 42.08% of the women were aged between 18 to 20 years, 45.70% of the mother had primary education and 35.75% of the mothers had secondary education and Majority (83.26%) belonged to Hindu religion and 40.27% belonged to Class IV socio economic strata according to the Modified B G Prasad's classification.

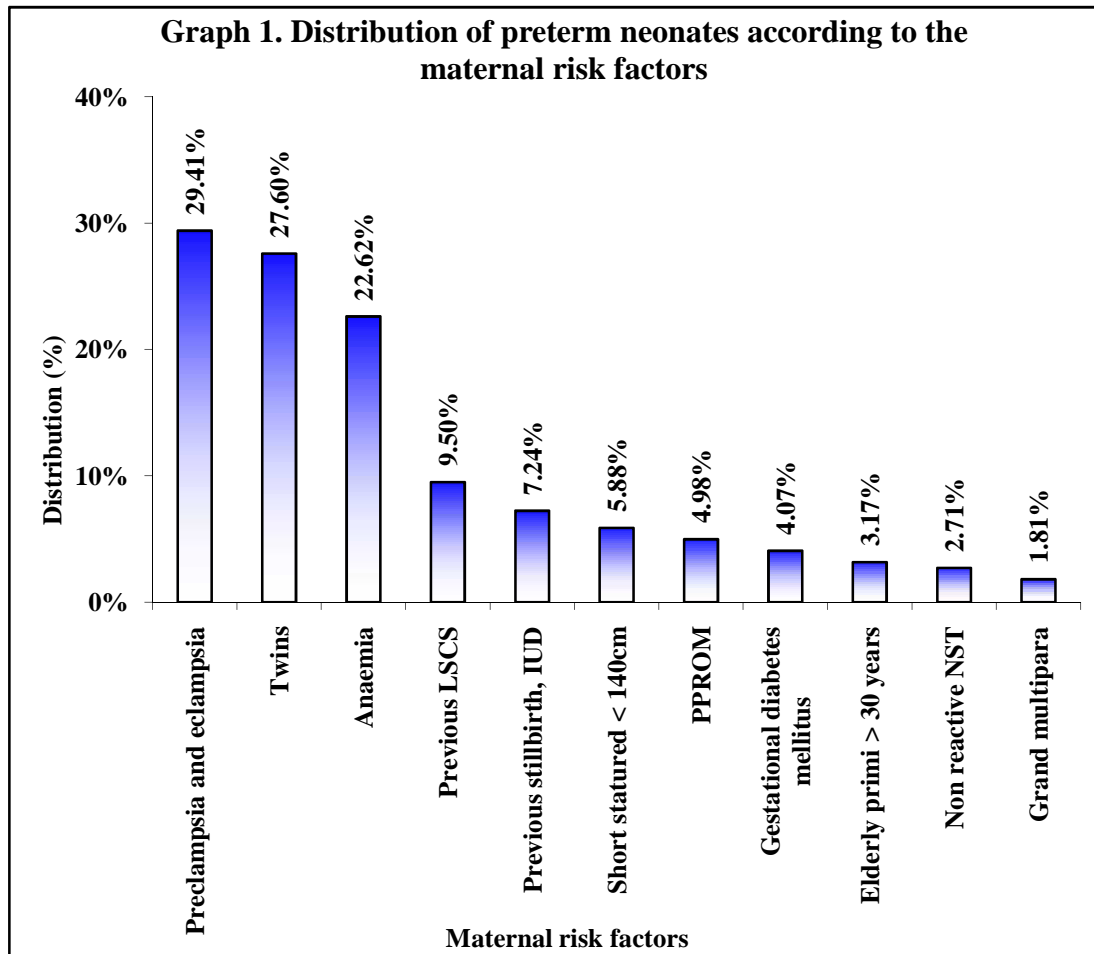
II. Maternal PREGNANCY Profile;
Table 2. Maternal pregnancy profile

Variables	Findings	Distribution (n=221)	
		Number	Percentage
Gravida	Primi	107	48.42
	2	76	34.39
	3	26	11.76
	4	12	5.43
Antenatal visits	2	27	12.22
	3	194	87.78
Antenatal scan	Yes	220	99.55
	No	1	0.45
Consanguinity	Consanguineous	83	37.56
	Non-Consanguineous	138	62.44

In this study, 48.42% of the mothers whose neonates received PDHM were primigravida, 87.78% had three antenatal visits, 99.55% of the mothers had undergone antenatal scans and history of consanguineous marriage was noted in 37.56%.

Table 3. Antenatal risk factors for preterm birth

Risk factors	Distribution (n=221)	
	Number	Percentage
Elderly primi > 35 years	7	3.17
Short statured < 145 cm	13	5.88
Preeclampsia and eclampsia	65	29.41
Anaemia	50	22.62
Gestational diabetes mellitus	9	4.07
Previous stillbirth, IUD	16	7.24
Previous LSCS	21	9.50
Grand multipara	4	1.81
PPROM	11	4.98
Non reactive NST	6	2.71
Twins	61	27.60



In the present study, the commonest risk factors for preterm birth noted were pre-eclampsia and eclampsia (29.41%), followed by twin pregnancy (27.60%) and anemia (22.62%)

III. BIRTH HISTORY

Table 4. Clinical profile of preterm neonates

Variables	Findings	Distribution (n=221)	
		Number	Percentage
Mode of delivery	NVD	69	31.22
	LSCS	152	68.78
	Instrumental	2	0.90
Gestational age (weeks)	<28	4	1.81
	28 to 34	97	43.89
	>34	120	54.30
Modified gestational age by Ballard score (weeks)	<28	0	0
	28 to 34	147	66.52
	>34	74	33.48

In the study, 68.78% of the neonates who received PDHM were born through LSCS, 54.30% had gestational of age >34 weeks and 66.52% had modified gestational age of between 28 to 34 weeks by Ballard score.

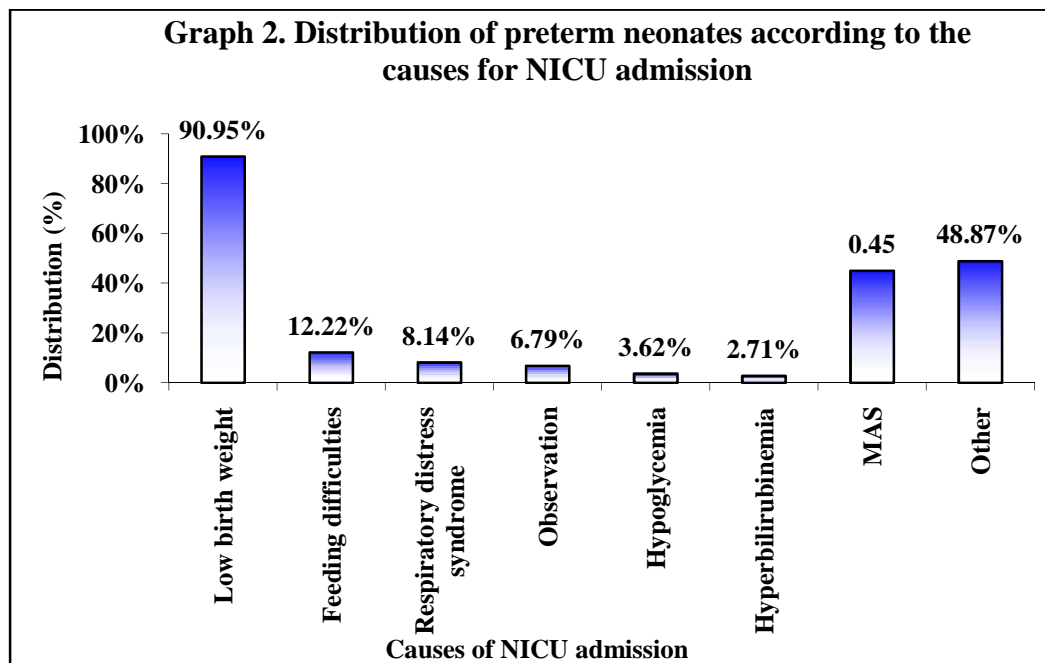
Table 5. Birth characteristics of preterm neonates receiving PDHM

Parameters	n	Mean		Median	IQR	Range	
		Mean	SD			Min	Max
Age (hours)	221	3.65	7.37	110.75	3.50	0.50	72.00
Modified ballard score	221	33.52	1.94	126.50	3.00	28.00	37.00
APGAR score at 5 minutes	218	7.65	0.80	113.00	1.00	4.00	9.00

The descriptive data regarding age, gestation age as per Modified Ballard score and APGAR score at five minutes of the neonates who received PDHM is as shown in Table 14. APGAR score of 3 preterm neonates were not known ,since they were delivered in outside hospital.

Table 6. Distribution of preterm neonates according to the causes for NICU admission

Causes of NICU admission	Distribution (n=221)	
	Number	Percentage
Low birth weight	201	90.95
Respiratory distress syndrome	18	8.14
MAS	1	0.45
hyperbilirubinemia	6	2.71
Feeding difficulties	27	12.22
Observation	15	6.79
Hypoglycemia	8	3.62
Kangaroo mother care	73	48.87



In the study ,themost common cause for admission to the NICU was LBW along with multiple factors as noted in the above table.

IV. ANTHROPOMETRIC PROFILE OF PRETERM NEONATES

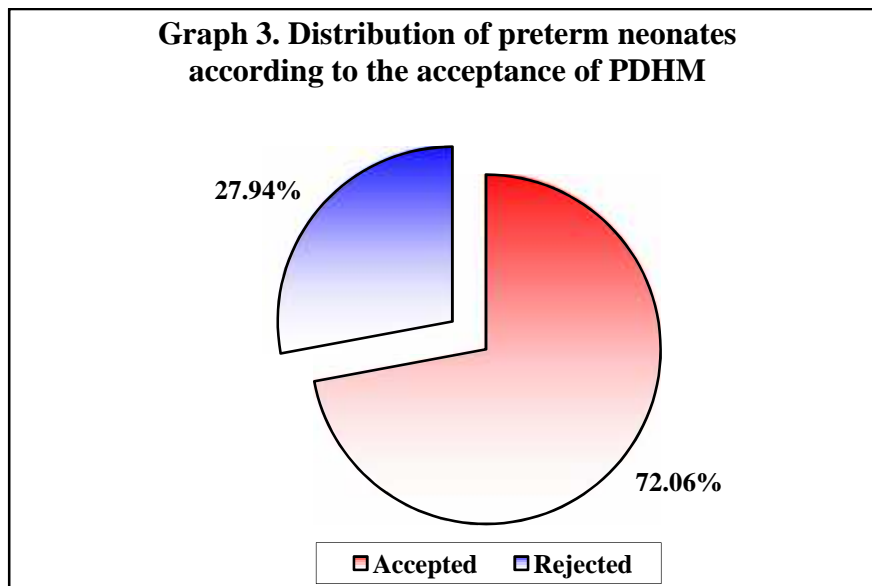
Table 7. Anthropometric profile of preterm neonates receiving PDHM at admission

Parameters	n	Mean		Median	IQR	Range	
		Mean	SD			Min	Max
Weight (Kg)	221	1.81	0.43	111.50	0.55	0.61	3.70
Length (cms)	221	41.98	2.66	131.50	3.00	32.00	48.00
Head circumference (cms)	221	29.57	1.75	125.50	2.00	24.00	34.00

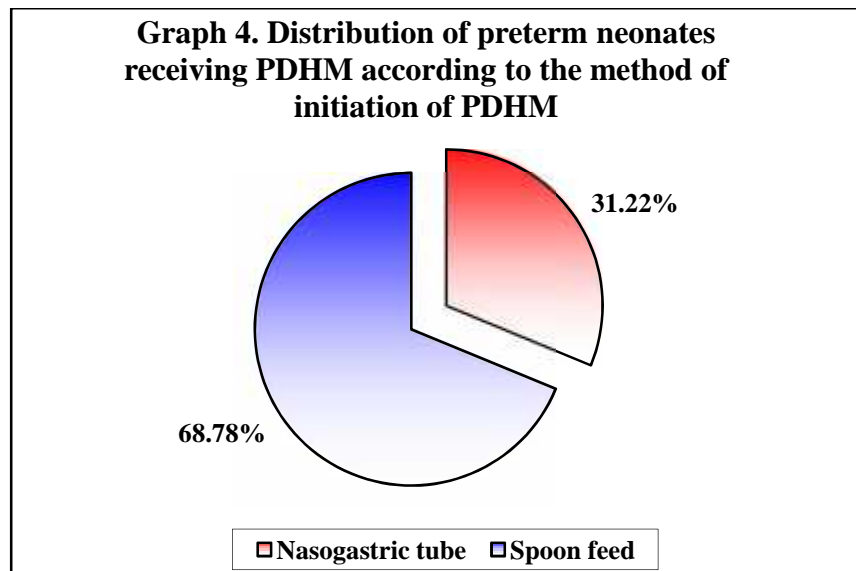
The descriptive data Anthropometry of the neonates who received PDHM is as shown in Table 7.

Of the 221 neonates mean weight was 1.81 ± 0.43 kg , length being 41.98 ± 2.66 cms , and mean head circumference being 29.57 ± 1.75 cm

V. FEEDING HISTORY AND DETAILS



In the study, out of 433 neonates who were offered PDHM, 312 (72.06%) accepted PDHM. The common cause of non-acceptability of PDHM among 121 neonates were; parental refusal (57.85%), non-acceptability of donor milk by grandparents (29.75%), fear that the donor milk can cause disease to preterm neonate (12.40%), and social factors like caste and religion(5.31%).



In this study 68.78% of the neonates were initiated on PDHM by nasogastric method.

Table 8. Mean duration and average consumption of PDHM

Parameters	n	Mean		Median	IQR	Range	
		Mean	SD			Min	Max
Total duration of PDHM given (Days)	221	5.19	3.93	113.00	3.00	1.00	28.00
Average consumption of PDHM (mL)	221	34.93	14.80	121.70	20.60	6.00	87.94

The mean duration to PDHM feeding was 5.19 ± 3.93 days and average consumption of PDHM was 34.93 ± 14.80 mL / day

VI. INCIDENCE OF NEC

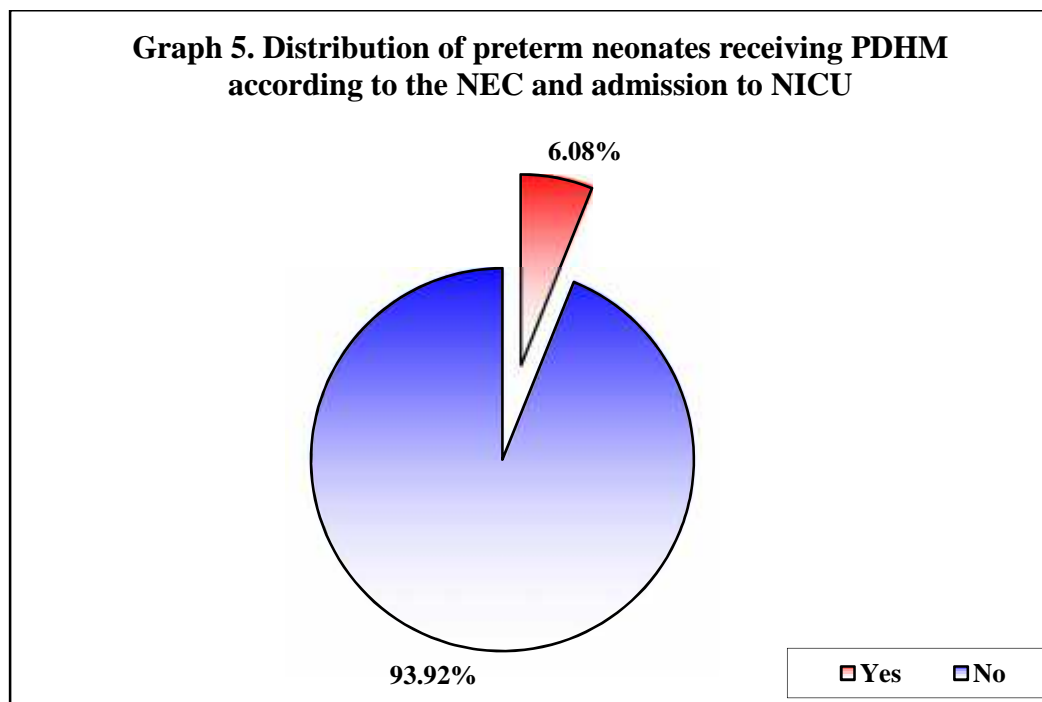
Table 9. Incidence of NEC

NEC	Distribution (n=221)	
	Number	Percentage
Yes	9	4.07
No	212	95.93
Total	221	100.00

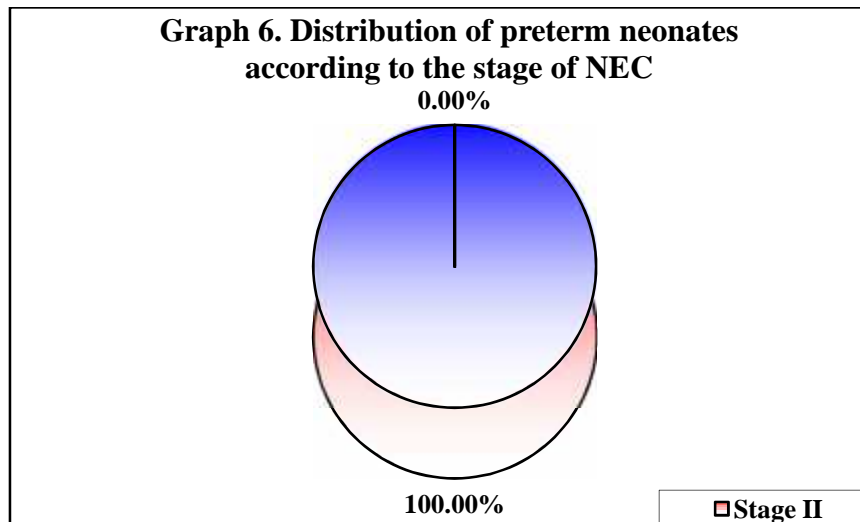
In the present study, 9 neonates (4.07%), of the 221 neonates who received PDHM, including 73 who were stable initially and receiving only KMC care in the special KMC ward situated within NICU, developed NEC.

Table 10. Distribution of preterm neonates receiving PDHM according to the NEC and admission to NICU

NEC	Distribution (n=148)	
	Number	Percentage
Yes	9	6.08
No	139	93.92
Total	148	100.00

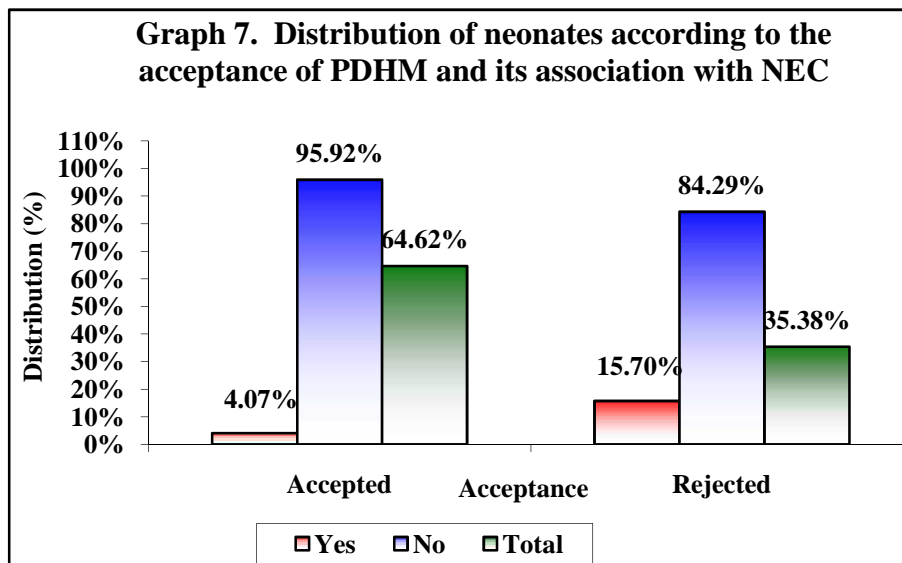


In the present study, 9 neonates (6.08%) out of 148 preterm neonates who were unstable initially requiring NICU care and receiving PDHM later, developed NEC.



In the present study all the nine neonates who developed NEC had stage II NEC.

Distribution of neonates according to the acceptance of PDHM and its association with NEC



p<0.001

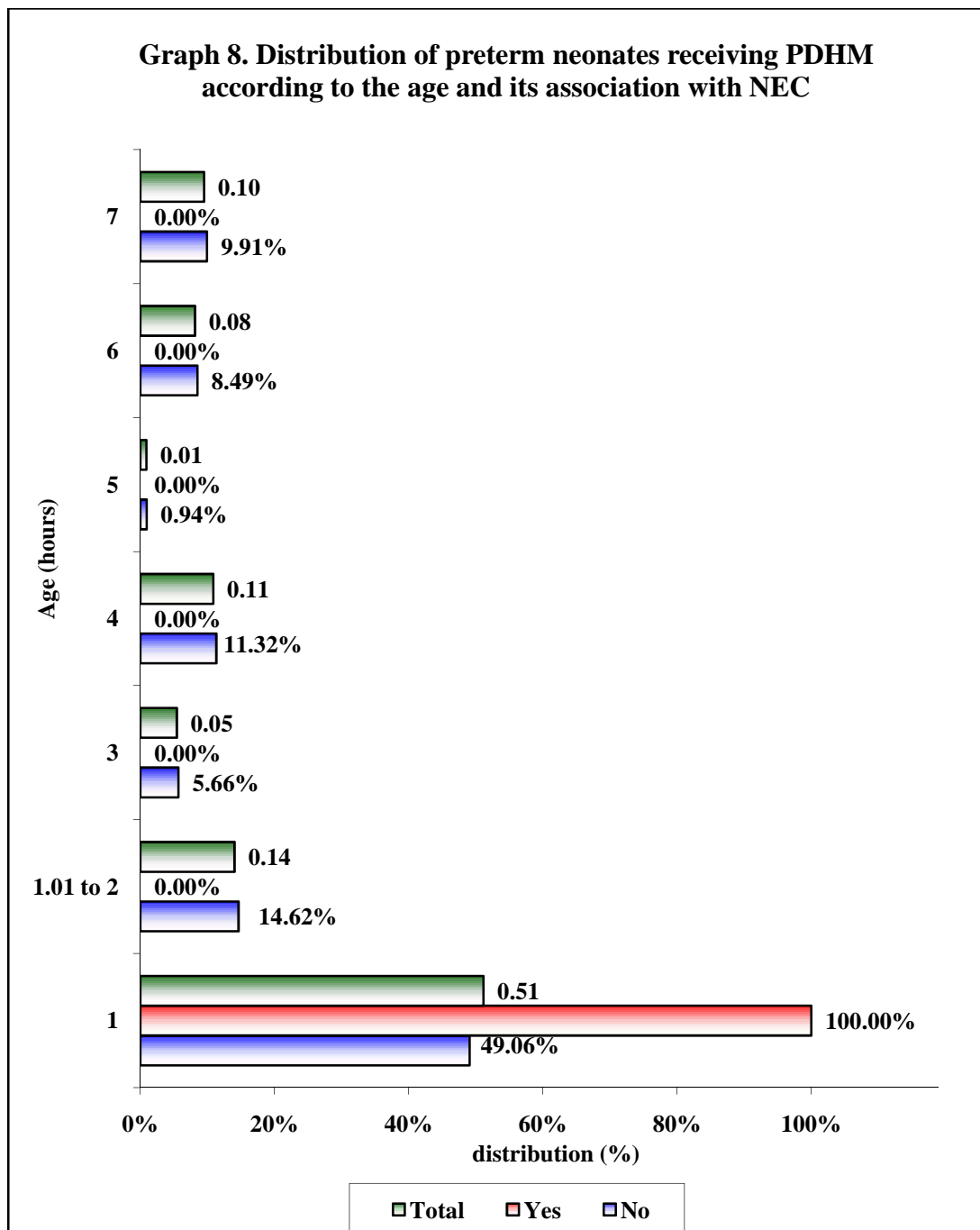
In the present study the frequency of NEC was significantly low among the neonates who received the PDHM compared to those who did not receive PDHM (4.07% vs 15.70%; p<0.001).

Table 11. Distribution of preterm neonates receiving PDHM according to the Gender and its association with NEC

Sex	NEC				Total	
	No		Yes		No.	%
	No.	%	No.	%		
Boys	121	57.08	3	33.33	124	56.11
Girls	91	42.92	6	66.67	97	43.89
Total	212	100.00	9	100.00	221	100.00

p = 0.144

In this study 56.11% of the neonates were boys and 43.89% were girls. The boy to girl ratio was 1.27:1. However no association was found between NEC and sex of the neonate (p=0.144).



p=0.348

In the present study 51.13% neonates were aged 1 hour at the time of admission to NICU. However no association was found between NEC and age of the neonate (p=0.348).

Table 12. Distribution of preterm neonates receiving PDHM according to the socio-economic status and its association with NEC

Socio economic status	NEC			
	No		Yes	
	No.	%	No.	%
Class I	8	3.77	4	44.44
Class II	38	17.92	2	22.22
Class III	72	33.96	2	22.22
Class IV	88	41.51	1	11.11
Class V	6	2.83	0	0.00
Total	212	100.00	9	100.00

p = 0.001

In this study maximum frequency of NEC was noted in neonates who belonged to class I socio economic strata (44.44%) compared to none of the neonate with Class V socio economic strata (0%) (p=0.001).

Table 13. Distribution of preterm neonates receiving PDHM according to the mother's gravida and its association with NEC

Gravida	NEC			
	No		Yes	
	No.	%	No.	%
Primi	102	48.11	5	55.56
2	74	34.91	2	22.22
3	25	11.79	1	11.11
4	11	5.19	1	11.11
Total	212	100.00	9	100.00

p = 0.689

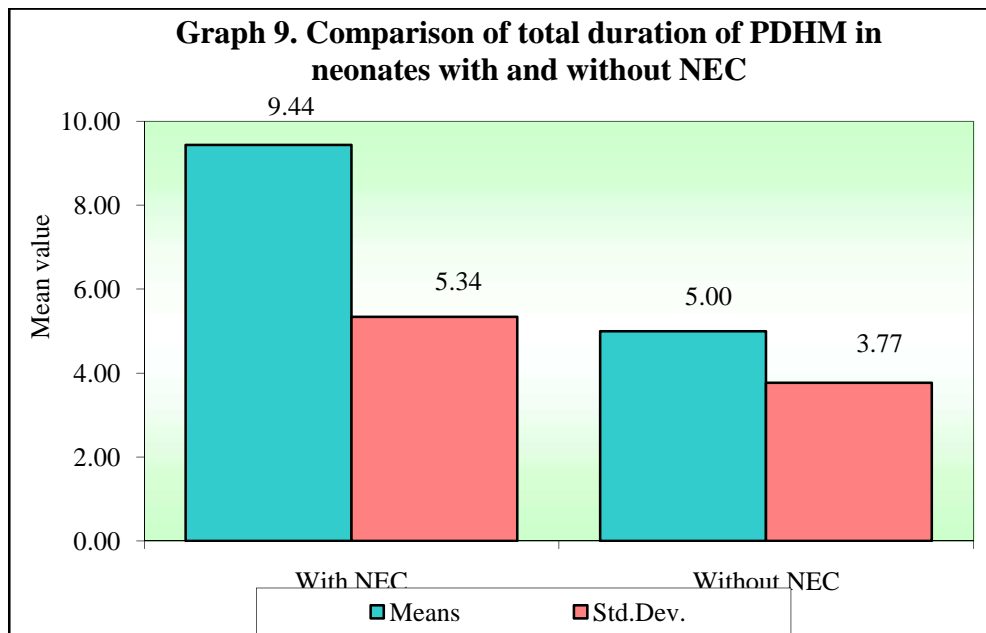
In the present study no association was found between NEC and gravida of the mother (p=0.689).

Table 14. Comparison of birth weight, modified Ballard score and APGAR score in neonates receiving PDHM with and without NEC

Parameters	NEC				p value
	Yes (n=9)		No (n=139)		
	Median	IQR	Median	IQR	
Birth weight	1.10	0.16	1.70	0.50	0.029
Modified Ballard score	29.00	2.00	33.00	2.00	0.040
APGAR score	7.00	1.50	8.00	1.00	0.069

In this study the median birth weight ($p=0.029$) and modified Ballard score (0.040) were significantly low in children with NEC but APGAR score was comparable ($p=0.069$).

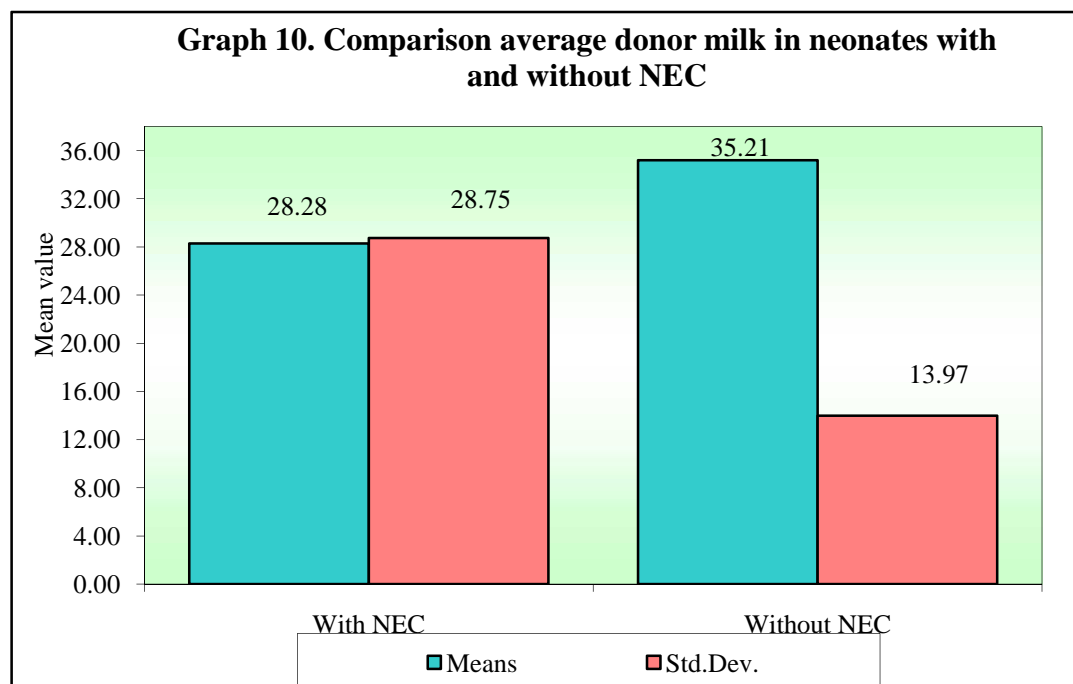
Comparison of total duration of PDHM in neonates with and without NEC



In the present study the total duration of PDHM was significantly high among the neonates with NEC (9.44 ± 5.34 vs 5.00 ± 3.77 days; $p < 0.001$).

Table 15. Comparison average donor milk in neonates with and without NEC

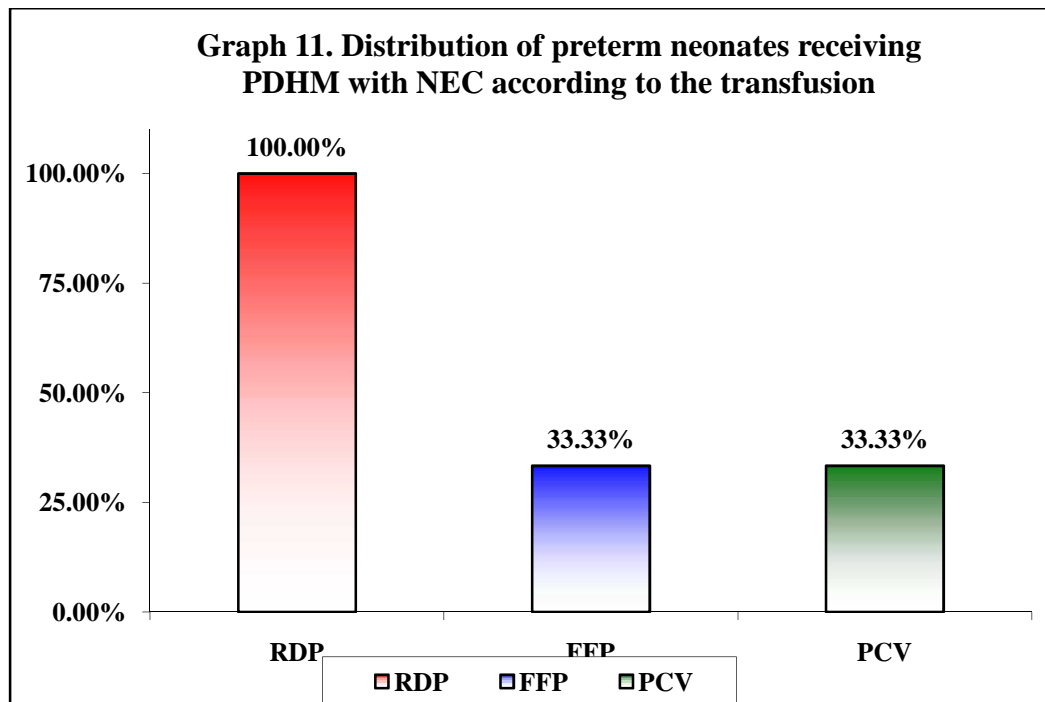
NEC	Means (mL/day)	Std. Dev.
With NEC	28.28	28.75
Without NEC	35.21	13.97
Total	34.93	14.80
t-value		-1.3793
p-value		0.1692



In this study the consumption of average donor milk was similar in neonates with and without NEC (28.28 ± 28.75 vs 35.21 ± 13.97 mL; $p=0.169$).

Table 16. Distribution of preterm neonates receiving PDHM with NEC according to the transfusion

Transfusion	Distribution (n=9)	
	Number	Percentage
Randomized donor pletelet (RDP)	9	100.00
Fresh Frozen plasma (FFP)	3	33.33
Packed cell volume (PCV)	3	33.33

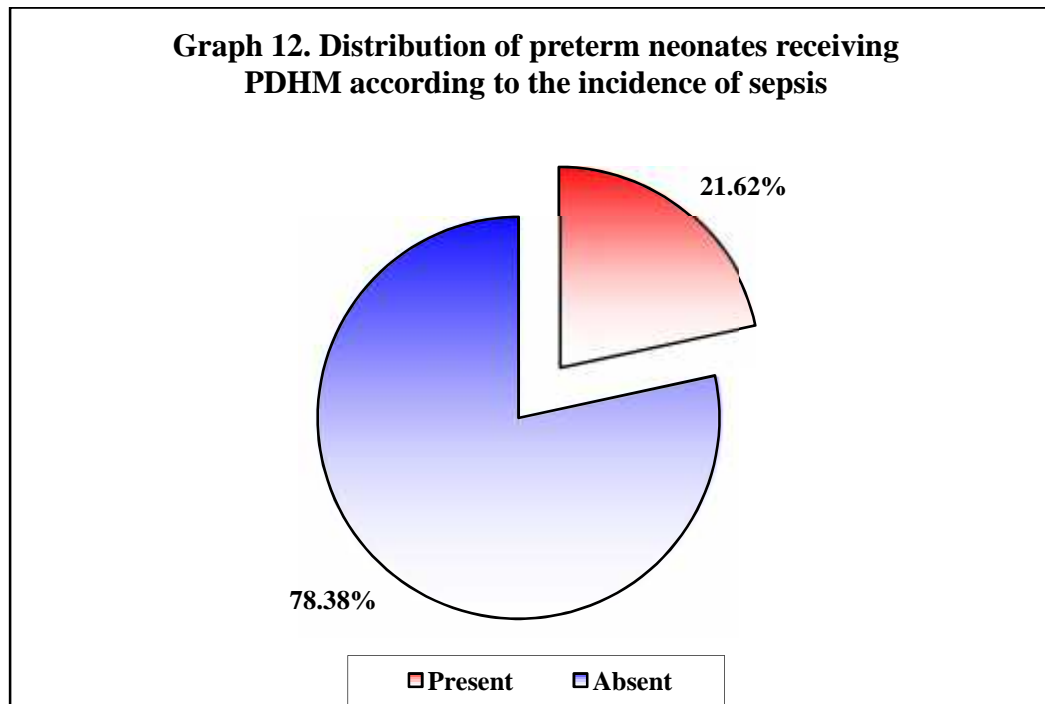


In the present study Nine neonates who developed NEC received RDP(100%)transfusion while 33.33% of the neonates each received FFP and PCV.

VII. SECONDARY OUTCOMES

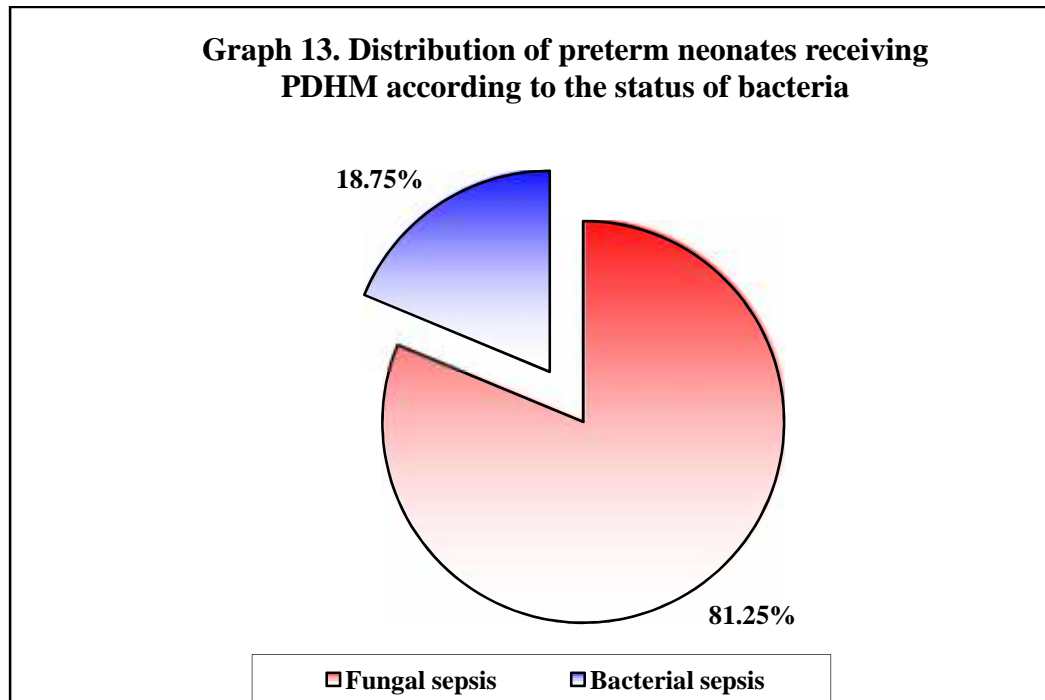
Incidence of Sepsis:

Distribution of preterm neonates receiving PDHM according to the NEC and admission to NICU and sepsis



In the present study 32 (21.62%) had blood culture proven sepsis.

Distribution of preterm neonates receiving PDHM according to the NEC, admission to NICU and status of bacteria



In this study of the 32 neonates with sepsis, 26 (81.25%) had fungal sepsis and 6 (18.75%) had bacterial sepsis it was associated with increased Highly sensitive C-reactive protein (HsCRP) values and leucopenia and thrombocytopenia .

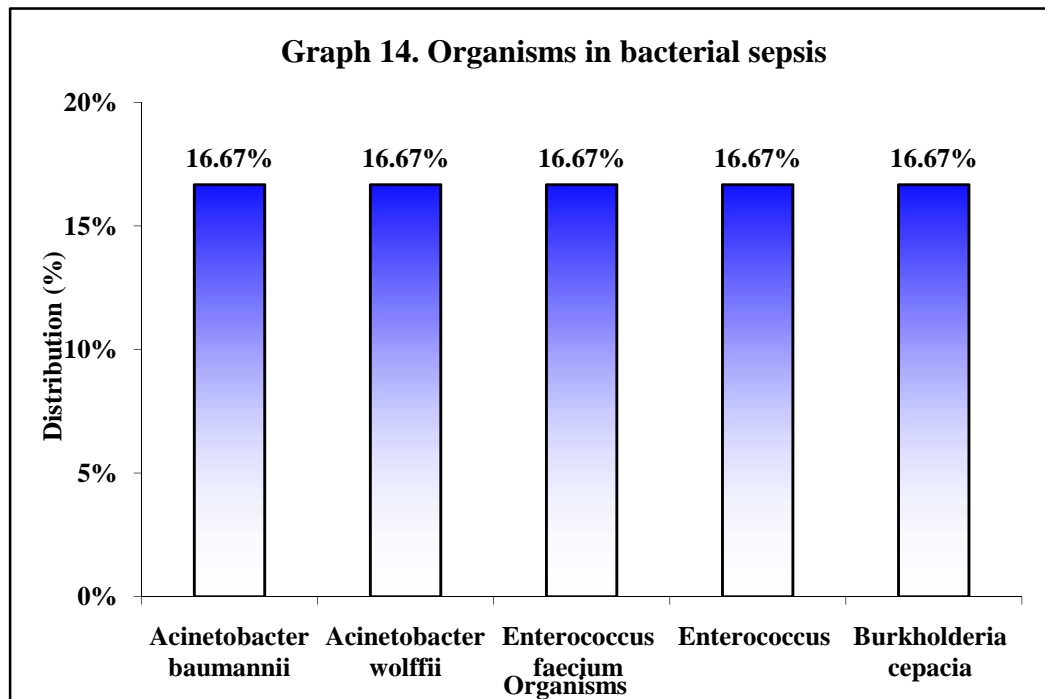
Table 17. Organisms in fungal sepsis

Organisms	Distribution (n=26)	
	Number	Percentage
Candida glabrata	22	84.62
Candida tropicalis	2	7.69
Candida Kruzi	1	3.85
Candida albicans	1	3.85

In the present study out of 26 neonates with fungal sepsis, candida glabrata was isolate in 22 neonates (84.62%), candida tropicalis in 2 neonates (7.69%) and one neonate each (3.85%) was infected with Candida kruzi, Candida albicans.

Table 18. Organisms in bacterial sepsis

Organisms	Distribution (n=6)	
	Number	Percentage
Acinetobacterbaumannii	1	16.67
Acinetobacterwolffii	1	16.67
Enterococcus faecium	1	16.67
Enterococcus	1	16.67
Klebsiellapneumoniae	1	16.67
Burkholderiacepacia	1	16.67
Total	6	100.00



In this study, out of 6 neonates with bacterial sepsis, one (16.67%) neonate each had *Acinetobacterbaumannii*, *Acinetobacterwolffii*, *Enterococcus faecium*, *Enterococcus*, *Klebsiellapneumoniae* and *Burkholderiacepacia*.

Table 19. Comparison of other parameters in NEC

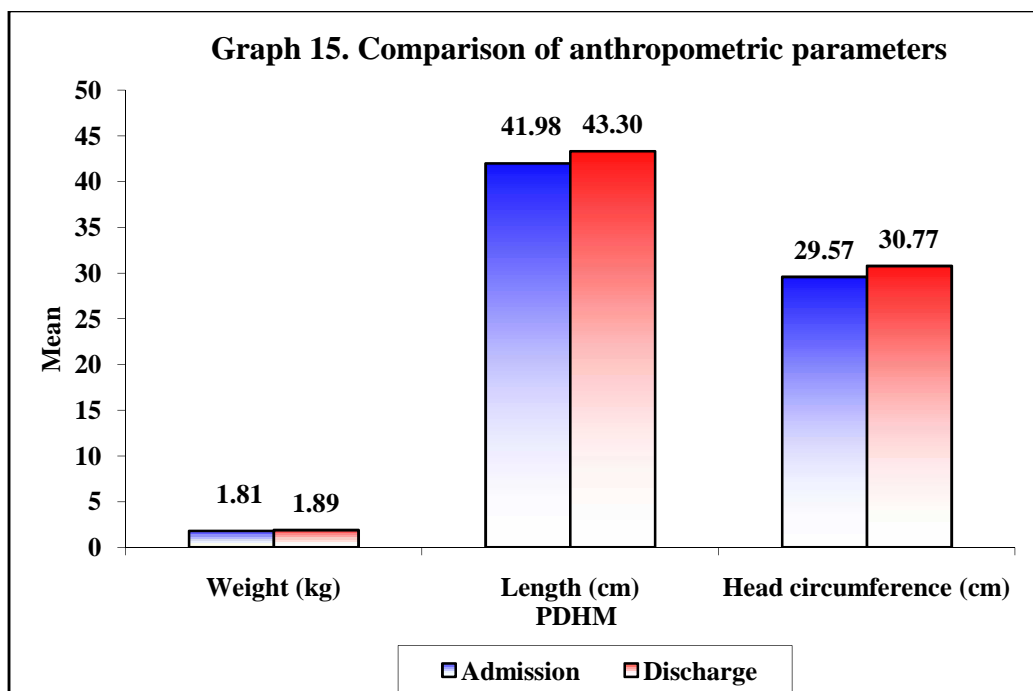
Parameters	NEC (n=221)				P value
	Yes		No		
	Mean	SD	Mean	SD	
Gestational age (weeks)	29.22	33.71	1.09	1.74	<0.001
Haemoglobin (gm%)	14.78	2.80	17.23	2.29	0.003
White blood cell count (per cumm)	7812.50	2870.81	10516.24	4010.89	0.067
Hs-CRP	45.23	29.73	17.42	19.82	0.003
Platelet count (per cumm)	102629.60	35853.65	190660.30	80269.24	0.001
NICU stay (Days)	25.77	4.52	8.59	8.32	<0.001
NICU+KMC stay (Days)	1.00	0.00	1.34	1.49	0.743
KMC duration (Days)	9.00	2.82	6.93	2.83	0.306
Duration of hospital stay	25.77	4.52	8.59	8.32	<0.001

In the present study gestational age, Hs-CRP, duration of NICU stay and duration of hospital stay was significantly high ($p<0.050$) in neonates with NEC but haemoglobin levels were significantly low ($p=0.003$).

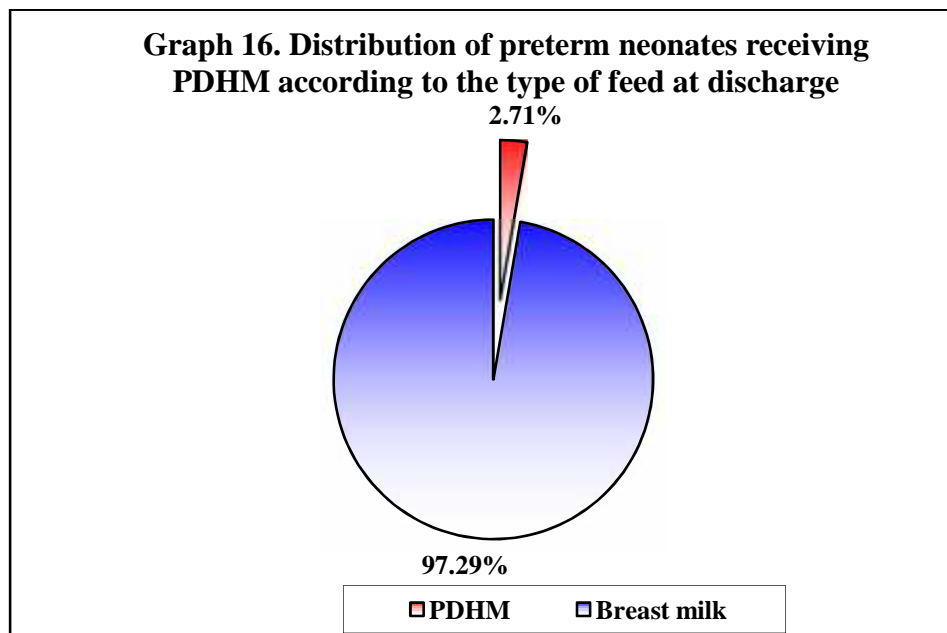
Out of 221 neonates with NICU admission, PDHM was continued in 6 babies at 28 days while, initiation of mothers milk was done in 215 neonates. The average age to start own mother’s milk among these 215 neonates was 4.22 ± 3.67 days.

Table 20. Comparison of anthropometric parameters from admission and at discharge

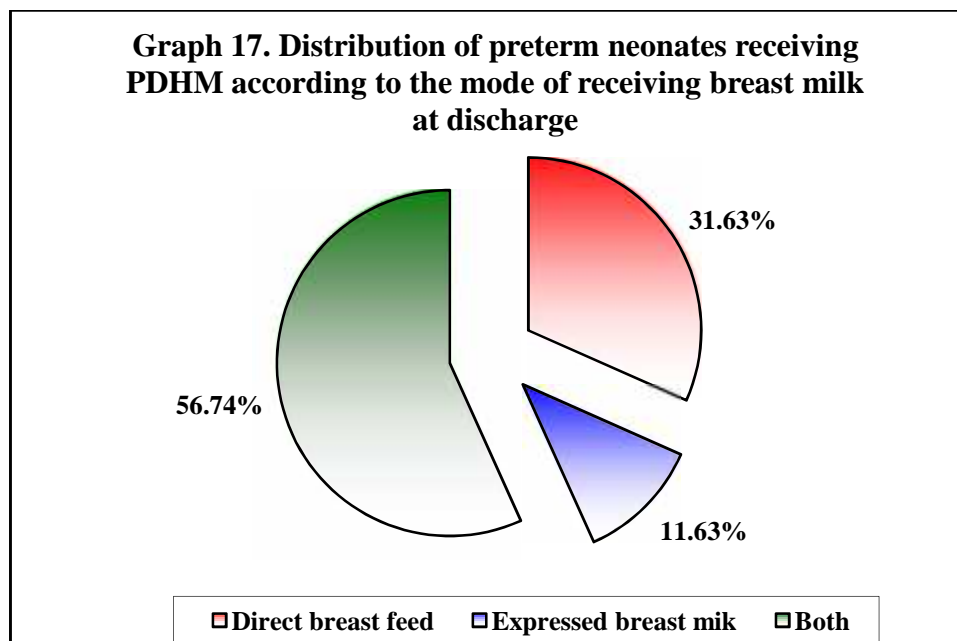
PDHM	Interval				% change	p value
	Admission		Discharge			
	Mean	SD	Mean	SD		
Weight (Kg)	1.81	0.43	1.89	0.42	4.61	<0.001
Length (cm)	41.98	2.66	43.30	2.32	3.15	<0.001
Head circumference (cm)	29.57	1.75	30.77	1.51	4.07	<0.001



In this study significant increase in weight (4.61%), length (3.15%) and head circumference was (4.07%) noted at discharge ($p < 0.001$).



In the present study, Majority (97.29%) of the neonates were on breast milk and 2.71% were on PDHM at discharge.



In this study of the 215 neonates on breastmilk, 122 (56.74%) were on direct breast feed as well as on expressed breast milk and 68 (31.63%) were on direct breast milk and 25 (11.63%) were on expressed breast milk.

DISCUSSION

Necrotizing enterocolitis (NEC) and sepsis are the two devastating medical conditions seen in the preterm neonates associated with increased morbidity and mortality. Human milk protects premature infants from NEC and sepsis. Under some circumstances, when mothers own milk is not available, pasteurized donor human milk (PDHM) fills the gap as recommended by WHO.⁸ The present study was an attempt to find out the incidence of NEC among preterm neonates receiving PDHM, to assess acceptability of PDHM in Recipients and to evaluate the outcomes among preterm neonates receiving PDHM with respect to incidence of sepsis, mortality rate, duration of NICU stay, growth parameters (weight gain, length, head circumference) and time of initiation of breastfeeding.

The cross-sectional study was conducted from January 2019 to February 2020 in the Department of Pediatrics, KAHER Jawaharlal Nehru Medical College and Hospital, Belagavi. During the study period a total of 1854 neonates were admitted in NICU of which, 634 were preterms. Among them, 433 preterm neonates were offered PDHM as other 201 were on breastfeed. Of these 433 preterms; 312 accepted PDHM which were enrolled in study of which 91 were excluded and 221 were analysed.

General characteristics:

In the study, majority of the mothers of the preterm neonates receiving PDHM were in the age group between 18-25 years. Similar findings of mothers being less than 25 years was reported by Melwani V.⁸² (55.7%) Trivedi P. et al.⁸³ (48.2%) Rashmi A et al.⁸⁴ (59.14%) and Rajgopal VM et al.⁸⁵ (71.4%). On the contrary, a recent Indian study to outline the morbidity and mortality pattern of preterms admitted

to NICU of a tertiary care center in Western Uttar Pradesh (UP)⁸⁶ reported 39.8% preterms born to mothers were less than 35 years.

In our study, 45.7% mothers had primary education. Similar observations regarding educational status of the mothers was reported (38.3%) in an Indian study⁸⁷ analyzing socio-economic factors influencing maternal motivation and willingness to donate the breast milk. However, another study from Karnataka reported 56.80% of mothers completed high school education.⁸⁴

Majority of the mothers of the preterm babies receiving PDHM in the study (83.26%) belonged to Hindu religion, which was consistent with regional sociodemographic profile (84.49% Hindu) of the study area.⁸⁸

In our study, most of the mothers (40.27%) belonged to Class IV socio economic strata according to the Modified B G Prasads classification,⁷³ which is similar to other Indian studies conducted to explore the risk factors for preterm birth and sociodemographic profile of the mother.^{83,87}

Our study reported, 48.42% of mothers of the preterm neonates, were primigravida. Similar observations were reported by various Indian Studies.⁸⁹⁻⁹²

In the present study, history of consanguineous marriage was present in more than one third of the women (37.56%). Similar observation of 39.2% of consanguineous marriage was reported in a South Indian study.⁹³ The overall prevalence of consanguineous marriage in India is 9.9%, with 23% in Southern region as reported by the National Family Health survey 2015-2016.⁹⁴

In the study, the commonest risk factor for preterm birth was pre-eclampsia and eclampsia (29.41%). These observations were consistent with other studies conducted to assess the risk factors for preterm birth,^{86,92,95-97} PIH leads to placental dysfunction leading to preterm birth.⁹⁸ Other common maternal risk factors for preterm delivery noted in the study were anemia and multiple gestation. Multiple gestation leading to preterm birth (27.6%) as observed in our study could be due to increased secretion of mediators such as corticotrophin releasing hormone (CRH) from the larger placental mass, and factors produced by the maturing fetal lung such as surfactant protein-A, which stimulates myometrial contractility and may contribute to preterm parturition.⁹⁹ We observed anemia in mothers (22.62%) as a risk factors for preterm delivery. Similar findings of preterm birth and LBW common in anemic mothers is reported in several studies.^{84,96,97,100} Mechanism explained as Iron deficiency increases oxidative damage to fetoplacental unit leading to preterm birth.^{100,101}

With regard to the birth history of the neonate, majority of the neonates (68.78%) were born through LSCS. Since our hospital is a tertiary care centre, high risk mothers with multiple complications are referred for better management and outcome leading to increased incidence of LSCS than vaginal delivery. Similar observations are reported in various studies.^{86,102}

Majority of the preterm neonates receiving PDHM assessed by modified ballard score in the study, were was between 28-34 weeks (66.52%) with the mean gestational age of 33.52 ± 1.94 weeks. Similar observation is reported by Adhisivam et al.⁶² (31.8 ± 2 weeks) and Debora et al.¹⁰³ (29.5 ± 2.3 weeks).

In the present study, most of the premature neonates were males (56.11%) with male to female of 1.27:1. Hassan N. et al. (2019) in his study to outline the morbidity and mortality pattern of preterms admitted to NICU of a tertiary care center in Western Uttar Pradesh (UP) reported that, 74.5% of neonates being boys. Similar gender distribution pattern has been reported earlier in several other studies.¹⁰⁴⁻¹⁰⁶

In our study, the common cause for NICU admission was prematurity and its related complications namely low birth weight, feeding difficulties, respiratory distress syndrome, hypoglycemia, meconium aspiration syndrome. These observations are known and reported in several studies.¹⁰⁷⁻¹⁰⁹

INCIDENCE OF NEC

“The Incidence of NEC varies between 0.3-2.4% in every 1000 live births.³ In most centres, the incidence of NEC is between 1-5 % of all neonatal intensive care unit (NICU) admissions and 5-10% of very low birth weight (VLBW) infants (<1500 gm). At present, NEC is thought to develop in the premature babies in the setting of bacterial colonization, often after administration of non-breast milk feeds.”^{5,6,7}

Several studies^{11,14,56} have demonstrated that preterm neonates fed with DHM have a reduced risk, incidence and severity of NEC. Similar observation was noted in our study with significantly lower incidence of NEC among preterm neonates receiving PDHM (4.07%) and further it was significantly lower in sicker Preterms who were admitted to the NICU (6.08%), The incidence of NEC in the preterm neonates who were non PDHM fed was significantly higher when compared to PDHM fed preterms in the study.(15.7%, p<0.001) .

Several studies^{66,68,69} have shown the positive effect of PDHM in lowering the incidence of NEC as observed in our study. Many studies have reported decreased incidence of NEC in the NICUs after DHM was made available. A large cohort study conducted to assess the impact of donor milk, comparing “the incidence of NEC before and after a change in practice providing DHM when MOM was unavailable from 22 NICUs in California, showed a decreased incidence of NEC from 5.7% to 2.9% (p=0.0006).⁶⁸

A meta-analysis conducted by Quigley and McGuire,⁶⁷ reported results from 6 RCTs comparing DHM vs formula feeding, shown that preterm neonates fed with DHM was associated with lower incidence of NEC(RR.2.77 ; 95% CI, 1.40-5.46).Similar observation of decreased incidence of NEC (13.5% vs 3.4%; p<0.001) was reported by Chowning et al.⁶⁹ following implementation of a DHM program.

Several other studies have reported the lower incidence of NEC in preterms receiving PDHM compared to formula feeds. A study by Sullivan S. et al.⁵⁸ (2010) on preterm infants, noted a 77% reduction in NEC. In preterms who were fed an exclusive human milk diet compared with those fed human milk supplemented with cow-milk-based infant formula products. A meta-analysis by “Boyd CA et al.⁵⁹, (2007) based on three studies found a lower risk of NEC in infants receiving donor breast milk compared with formula (combined RR 0.21, 95% CI 0.06 to 0.76)”. A study reported by “Arslanoglu S. et al.⁶⁵ (2013) showed that feeding preterm infants with DHM when compared to formula feeding, was associated with a decreased risk of NEC from 16% to 6%”.

Contrary to our observations, a south Indian study from a tertiary care teaching hospital, comparing “the effect of fortified PDHM versus unfortified PDHM” on the incidence of NEC reported no increase in incidence of NEC in fortified PDHM group compared to unfortified PDHM group (2.5 versus 7.5%, $p=0.31$). The study concluded that standard fortification of PDHM does not increase the incidence of NEC among preterm neonates.⁶²

The frequency of NEC, in the present study was significantly low among the neonates who received the PDHM compared to those who did not receive PDHM (4.07% vs 15.70%; $p<0.001$) which was consistent with previous studies.^{9,62,64-66,68,69} stating the positive role of human milk in the prevention of NEC compared to formula feeding.

These observations hypothesize that, use of human milk (maternal or donor) may offer protection against NEC when compared to formula feeding among preterm neonates and helps in promoting breast feeding. Donor milk contains Human milk oligosaccharides which withstands the low pH of infants stomach and reaches distal small intestine and colon where it acts as a prebiotic and helps beneficial bacteria to thrive alongwith suppressing harmful bacteria , it also has anti-adhesives that blocks the attachment of viral and bacterial pathogens thus counteracting dysbiosis at the early stage of pathogenesis of NEC.¹¹⁰ This being the first study done in India where incidence of NEC is assessed with PDHM ,these observations require further validation due to lack of consistent data in the literature as impact of PDHM and NEC rates.

In our study group neonates who developed NEC had mean gestation age of 29 ± 2 weeks vs non-NEC neonates 33 ± 2 weeks being statistically significant (p value 0.040), prematurity being the biggest risk factor for development of NEC as being well known and proven in various studies^{3,4,5,6,7}

Nine neonates who developed NEC in our study had mean birth weight of 1100 ± 160 gm vs non-NEC neonates 1700 ± 500 gm being statistically significant (p value 0.029) is similar to various studies stating NEC being more common in VLBW and ELBW babies^{3,4,5,6,7,9,19,12,64}.

Secondary outcomes

Acceptability Of PDHM:

The rate of acceptability of PDHM in the study was 72.06% which is similar to that reported from a western study done by Ward et al (77%).¹¹¹ However a higher rate of acceptability (85.4%) was reported by an Indian study done by Melwani et al.⁸² On the contrary studies from Ethiopia¹¹² and Nigeria¹¹³ have reported lower rates (15.2% and 13.1%) acceptability of PDHM by the mothers. The commonest reason for non-acceptability of the PDHM in our study was parental refusal, However reason stated in other studies for non-acceptability of PDHM were fear of transmission to disease to the baby,¹¹¹⁻¹¹³ cultural fear relating to caste and creed.^{82,112}

The mean gestation of preterm neonates enrolled in our study was 33 ± 2 weeks, 68.78% of these neonates were started on donor milk with spoon feeds as per the recommendations of WHO on feeding of LBW infants⁸ as it helps in reaching full enteral feeds and early hospital discharge as compared to NG method of feeding preterms.^{114,115}

The mean duration of PDHM fed to the preterm neonates in the study was 5.19 ± 3.93 days with average consumption of 34.93 ± 14.80 ml/day. This is similar to an Indian study by Adhisivam et al.⁶² with mean duration of PDHM being 8.5 ± 5.1 days and 10 (5,14) days in study done by Alyahya et al from UK (2019).¹¹⁶ In another study done by Battersby et al.¹¹⁷ reported a mean duration of 8.5 (5.6,11.4). Indian study from Rajasthan reported average consumption of 95 ml of donor milk in the neonates,¹¹⁸ Italian study⁶⁸ reported average intake of donor human milk being 34.9 ± 12.5 ml among VLBW neonates enrolled in the study. However an Australian study¹¹⁹ reported consumption of donor milk range being 3-9271 ml, it varied as the neonates enrolled were divided into four groups and one group received only donor milk till discharge to assess the outcomes.

Sepsis

The incidence of sepsis in the preterm neonates receiving PDHM in the study was 21.62%, which is similar to the incidence of sepsis (21.1%) reported by Deborah et al,¹⁰³ However, a higher incidence of sepsis in the preterms receiving donor human milk was reported by Adhisivam et al.⁶² (37.5%) and Corpeleijn et al.¹¹⁹ (36.6 %). Several other studies has shown a low incidence of sepsis ranging from 1-15%, compared to our observation.¹²⁰⁻¹²³ This higher incidence of sepsis in our study may be due to more number of sick neonates referred to our NICU which is a tertiary care, level two NICU.

The most common organism causing sepsis in the preterm neonates receiving PDHM in the study was fungus (26/148, 17.56%). This observation is similar to other studies by Yunus et al.¹²⁴ (15.7%) and Agarwal et al.¹²⁵ (13.6%) The most common fungus isolated in the study was *Candida glabrata* (22 neonates, 84.62%), followed by

Candida tropicalis (2 neonates, 7.69%) and *Candida kruzi* and *Candida albicans* (one neonate each, 3.85%). Similar findings are reported by ARTEMIS Antifungal Surveillance study¹²⁶ done between June 1997 and December 2007 in 41 countries who reported emergence of non-*albicans* species in fungal septicemia and several other studies.¹²⁷⁻¹³⁰

In the remaining (6 neonates , 18.75%) preterms neonates receiving PDHM bacterial sepsis was noted with organisms being *Acinetobacter baumannii*, *Acinetobacter wolffii*, *Enterococcus faecium*, *Enterococcus*, *Klebsiella pneumoniae* and *Burkholderia cepacia* (16.67% each).Further, Hs-CRP, Hb and platelet count in the neonates who developed NEC was clinically significant ($p < 0.050$) as reported in literature.^{3,4}

Neonatal mortality

In our study no neonatal mortality was noted among neonates who were enrolled and PDHM was given, Further Nine neonates who developed NEC were managed medically thus concluding PDHM not only lowers the incidence of NEC but also decreases the severity of the disease^{6,7,9,10,12,64,68}

Duration of NICU stay

The mean duration of NICU stay in neonates who developed NEC was 25.77 days with SD of 4.52 days vs Non-NEC 8.59 days with SD of 8.32 days, comparable to Adhisivam et al.⁶² with 15.2 days and SD being 7.2 days. It was observed that neonates who developed NEC required prolonged NICU stay as primary treatment being wait and watch with medical care and gradual watchful initiation of feeding^{3,4,5}.

Growth

In the study significant increase in weight (4.61%), height (3.15%) and head circumference was (4.07%) noted at discharge ($p < 0.001$) suggesting that PDHM promotes early postnatal growth. Study done by Deborah et al.¹⁰³ and Sisk et al.⁶⁶ reported no significant growth on donor human milk. On the contrary, several studies have reported that “Donor milk given as a sole diet is associated with a lower risk of NEC but slower growth in the early postnatal period”.^{12,59,70,71} The mechanism explained being pasteurization affecting the macro and micronutrients required for early postnatal growth.^{11,12} However in all the studies donor milk is used solely whereas in our study we switched to mothers milk as soon it was available. Further research is needed to confirm our findings and measure the effect of donor breast milk that is fortified or given as a supplement to mother’s own milk.

In our study out of 221 neonates who were initiated on PDHM, 215 were on breastfeeds at the time of discharge/28 days, of which 122 (56.74%) were on direct breast feed, 68 (31.63%) were on expressed breast milk and 25 (11.63%) were on both direct and expressed breast milk, with mean initiation of breastfeeding being 4.22 ± 3.67 days which is similar to study done by Adhisivam et al.⁶² with mean being of 6.5 ± 4.5 days.

The common concern in the centres where human milk bank is available is that, DHM might decrease breast feeding rates. In our study, most of the preterm on PDHM were on breast feeding either direct or expressed breast feed at the time of discharge. Similar observations reported by other studies shows that the presence of a human milk bank (HMB) and use of DHM in the NICU are associated with increased breast feeding rate at discharge.¹²⁹ Data from Italian association of Human milk banks

from 83 NICUs reports that exclusive breast feeding (EBF) rates significantly higher in NICUs with use of HMB as compared to NICUs without HMB.¹³¹ A study on impact of donor milk availability on preterm outcome in Californian NICUs, observed increased breast feeding rates (61.7%) at discharges. DHM availability fosters a breastfeeding friendly environment in which mothers are encouraged to attempt to produce their own milk and thereby increase the rate of breastfeeding.⁶⁸

Overall, the present study showed that both the incidence and the severity of NEC decreases with the use of PDHM, acceptability of PDHM in the study population was high despite few parents refused to accept the donor human milk based on social factors like caste and creed which needs to be overcome through awareness program regarding donor milk and milk banks by government and local institutions. Sepsis is important cause of morbidity among the preterm neonates despite on PDHM with fungal sepsis being common which can be attributed to prematurity itself and can be lowered down by following strict aseptic measures. Timely diagnosis and prompt management results in excellent outcome among the preterm neonates with NEC receiving PDHM with no mortality. Administration of PDHM also promotes early postnatal growth with no mortality. However, these findings require further validation due to potential limitations of the study.

Strengths

The strength of the study is that is the maiden study conducted to assess the benefits of PDHM in preterm neonates with special emphasis on NEC, promotion of breast feeding and post-natal growth and sepsis.

LIMITATIONS

The limitation of the study was that, the findings in this study were based on the data from a single centre and its study design being cross sectional ideally would have been RCT study. Also only short term outcomes were considered in the present study long term outcomes were not considered as it was beyond the scope of this study. Also the study evaluated only the attitude of mothers towards PDHM but the knowledge about the PDHM also needs to be assessed which is likely to help in promoting the PDHM.

Recommendations

Multicentric studies with longitudinal design involving large sample size with assessment of knowledge of the mothers towards PDHM and long term outcomes on growth and neurodevelopment may provide the feasibility of PDHM. Also studies comparing the NEC rates in formula feeding and PDHM may extend the true effect of PDHM in the prevention of NEC among the preterm neonates.

CONCLUSION

This case control study was conducted among preterm neonates receiving pasteurized donor human milk (PDHM) during the period of January 2019 to February 2020. The present study shown a decreased incidence of NEC among preterm neonates receiving PDHM. The rate of acceptability of PDHM was high. Sepsis was low with no mortality in neonates receiving PDHM. There was a positive effect of PDHM on growth of these neonates and breastfeeding at discharge, suggesting that PDHM promotes early postnatal growth and breastfeeding. Multicentric, larger sample size RCT studies are recommended to assess the effect of PDHM on NEC, sepsis and growth parameters .

SUMMARY

Necrotizing enterocolitis (NEC) and sepsis are the two devastating medical conditions seen in the preterm neonates. Human milk protects premature infants from NEC and sepsis. Under some circumstances, when mothers own milk is not available, pasteurized donor human milk (PDHM) fills the gap. The present study was an attempt to find out the incidence of NEC among preterm neonates receiving PDHM, to assess acceptability of PDHM in Recipients and to evaluate the outcomes among preterm neonates receiving PDHM with respect to incidence of sepsis, mortality rate, duration of NICU stay, growth parameters (weight gain, length, head circumference) and time of initiation of breastfeeding.

The cross-sectional study was conducted from January 2019 to February 2020 in the Department of Pediatrics, KLES Dr. Prabhakar Kore Hospital and Medical Research Centre, Belagavi. During the study period a total of 1854 neonates were admitted in NICU among them, 634 were preterms. Of this, 433 preterm neonates were offered PDHM as other 201 were on breastfeed. Out of 433 preterms; 312 accepted PDHM which were enrolled in study of which 91 were excluded and data pertaining to 221 neonates was available for analysis. The data was analysed and the important findings of the study are summarized as below.

- Out of 433 neonates who were offered PDHM, 312 (72.06%) accepted PDHM. The common cause of non-acceptability of PDHM among 121 neonates were; parents refusal (57.85%), non-acceptability of donor milk by grandparents (29.75%), fear that donor milk can cause disease to preterm neonate (12.40%), and social factors like caste and religion being (5.31%).

Further, among those neonates who received PDHM, most of the neonates (68.78%) were initiated through nasogastric tube. The mean duration of PDHM was 5.19 ± 3.93 days and average consumption of PDHM was 34.93 ± 14.80 mL per day.

- Among the infants who received PDHM, most of the mothers (42.08%) were aged between 18 to 20 years, had primary education (45.70%), majority of them (83.26%) belonged to Hindu religion and most of the mother's (40.27%) belonged to Class IV socio economic strata according to the Modified B G Prasad's classification.
- Most of the mothers whose neonates received PDHM were primigravida (48.42%), majority of the mothers (87.78%) had three antenatal visits, and had undergone antenatal scans (99.55%) while history of consanguineous marriage was noted in 37.56%.
- The commonest risk factor for preterm birth noted is being preeclampsia and eclampsia (29.41%).
- Majority of the neonates (68.78%) who received PDHM were born through LSCS, and had modified gestational age between 28 to 34 weeks by Ballard score (66.52%).
- The common cause for NICU Admission in neonates who received PDHM was low birth weight (90.95%).
- Of the total 221 preterm neonates enrolled in the study receiving PDHM 73 were stable and received KMC care in the special KMC ward situated within

NICU, 9 neonates (4.07%) developed NEC. Of the 148 neonates out of 221 with NICU care 9 neonates (6.08%) developed NEC.

- The frequency of NEC was significantly low among the neonates who received the PDHM compared to those who did not receive PDHM (4.07% vs 15.70%; $p < 0.001$).
- All the nine neonates who developed NEC had stage II NEC ; were managed medically and no neonate progressed to stage III NEC or required surgical intervention.
- Overall, 56.11% of the neonates were boys and 43.89% were girls. The boy to girl ratio was 1.27:1. However no association was found between NEC and sex of the neonate ($p = 0.144$).
- 51.13% neonates were aged 1 hour and no association was found between NEC and age of the neonate ($p = 0.348$).
- Maximum frequency of NEC was noted in neonates who belonged to class I socio economic strata (44.44%) compared to none of the neonate with Class V socio economic strata (0%) ($p = 0.001$).
- No association was found between NEC and gravida of the mother ($p = 0.689$).
- The median birth weight ($p = 0.029$) and modified Ballard score (0.040) were significantly low in children with NEC but APGAR score was comparable ($p = 0.069$).

- The total duration of PDHM was significantly high among the neonates with NEC (9.44 ± 5.34 vs 5.00 ± 3.77 days; $p<0.001$) and the consumption of average donor milk was similar in neonates with and without NEC (28.28 ± 28.75 vs 35.21 ± 13.97 mL; $p=0.169$).
- All the 9 preterm neonates who developed NEC (100%) received RDP transfusion, while three (33.33%) of the neonates each received FFP and PCV.
- Sepsis was noted in 32 (21.62%) neonates. Of the 32 neonates with sepsis, 26 (81.25%) had fungal sepsis and 6 (18.75%) had bacterial sepsis. Out of 26 neonates with fungal sepsis, *Candida glabrata* was isolated in 22 neonates (84.62%), *Candida tropicalis* in 2 neonates (7.69%) and one neonate each (3.85%) was infected with *Candida kruzi*, *Candida albicans*. Out of 6 neonates with bacterial sepsis, one (16.67%) neonate each had *Acinetobacter baumannii*, *Acinetobacter wolffii*, *Enterococcus faecium*, *Enterococcus*, *Klebsiella pneumoniae* and *Burkholderia cepacia*.
- Gestational age, Hs-CRP, NICU stay and duration of hospital stay was significantly high ($p<0.050$) in neonates with NEC but haemoglobin levels were significantly low ($p=0.003$). Out of 221 neonates with NICU admission, PDHM was continued in 6 babies while, initiation of mothers milk was done in 221 neonates. The average age to start the mother's milk among these 215 neonates was 4.22 ± 3.67 days.
- Significant increase in weight (4.61%), height (3.15%) and head circumference was (4.07%) noted at discharge ($p<0.001$).

- At discharge from NICU majority of the neonates (97.29%) were on breast milk and 2.71% were on PDHM. Of the 215 neonates on breast milk, 122 (56.74%) were on direct breast feed as well as on expressed breast milk and 68 (31.63%) were on direct breast milk and 25 (11.63%) were on expressed breast milk.

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ANNEXURE I – CONSENT FORM

CONSENT FOR PARTICIPATION IN RESEARCH

“Incidence of NEC among preterm neonates receiving Pasteurized donor human milk a one year cross sectional study at KLE Dr. PrabhakarKore hospital and MRC, Belagavi.”

Principal Investigator: _____

Co – investigator: _____

You have been asked to involve your child in the above said research to be conducted at NICU of KAHER JN medical college hospital, Belgavi by _____, PG student in the Department of Paediatrics at Jawaharlal Nehru Medical College, Belgavi.

Introduction

Purpose of the study

Participation of your child will help us to know the incidence of Necrotizing enterocolitis in preterm neonates receiving PDHM and other observational outcome at discharge. You are free to discontinue the participation in the study at any time for any reasons and you will not be paid any reimbursement for participation in the research. Hence involving your child in the study is your voluntary decision.

Voluntary participation

Your child’s participation in this study is your voluntary decision, whether or not to participate will not affect your current or future relationship with KLEs Dr. PrabhakarKore Hospital & MRC, Belgavi.

Risk and benefits

There are no risks involved.

Reduction in morbidity and mortality in the neonates.

Privacy and Confidentiality

The only people who will know that you are a research participant are member of the research team. No information about you or provided by you, during research will be disclosed to others without your written consent. When the results of the research are published or discussed in the conferences, no information will be disclosed that would reveal your identity. Any information obtained in connections with this study and that can be identified with you remain confidential and will be disclosed only with your permission.

Queries:

If you have any queries you may contact

If you have any questions about your rights or research participation you may contact

Chairman ethical committee:

DR. ROOPA. M.BELLAD MD DCH
PROFESSOR
DEPARTMENT OF PAEDIATRICS,
KAHER
JAWAHARLAL NEHRU MEDICAL COLLEGE,
BELGAVI-590010

You will be given a copy of this form for your information and to keep for your records

STATEMENT OF CONSENT

I hereby voluntarily agree for my participation in this study. I understand that even if I have the liberty to withdraw at any time. My signature below indicates that I have read or have been told in the language I understand , about this entire consent form including the risks and benefits and have had all my questions answered. I will be given a copy of this consent form.

Signature of the authorized representative/ parent: _____

Date: _____

Name: _____

Relation to the Subject: _____

Signature of the witness: _____

Date: _____

Name: _____

Signature of investigator: _____

Date: _____

Name: _____

ಸಂಶೋಧನೆಯ ಭಾಗವಹಿಸುವಿಕೆಗೆ ಒಪ್ಪಿಗೆ

"ವ್ಯವಹಾರವಿ ನಿಯೋಗಿಗಳ ನಡುವೆ ಎನ್‌ಎಸ್ ಫಲನೆಯು ಪಾಶ್ಚಾತ್ಯೀಕೃತ ದಾನಿ ಮಾನವ ಹಾಲನ್ನು ಒಂದು ವರ್ಷದ ಕ್ರಾಸ್ ವಿಭಾಗದ ಅಧ್ಯಯನವನ್ನು ಕೆ ಎಲ್ ಇ ಡಾ. ಪ್ರಬಾಕರ್ ಕೋಲೆ ಆಸಕ್ತಿಯಲ್ಲಿ ಮತ್ತು ಎನ್‌ಎಸ್ ಫಲನೆಯ ಯಲ್ಲಿ ಸ್ವೀಕರಿಸುತ್ತದೆ."

ಪ್ರಧಾನ ತನಿಖಾಧಿಕಾರಿ:

ಸಹ - ತನಿಖೆದಾರ: ಡಾ. ಸೌರಬ್, ಪಿಜಿ ವಿದ್ಯಾರ್ಥಿ

ಬೆಳಗಾವಿಯ ಐದಾಪರಲಾಲ್ ನೆಹರು ಮೆಡಿಕಲ್ ಕಾಲೇಜಿನಲ್ಲಿ ಪೀಡಿಯಾಟ್ರಿಕ್ಸ್ ಇಲಾಖೆಯ ಪಿ.ಜಿ. ವಿದ್ಯಾರ್ಥಿಯಾಗಿದ್ದ ಡಾ. ಸೌರಬ್ ಅವರು ಬೆಳಗಾವಿ ಕಾಪರ್ ಕೆಎಸ್ ಮೆಡಿಕಲ್ ಕಾಲೇಜು ಆಸ್ಪತ್ರೆಯಲ್ಲಿ ನಡೆಸಿದ ಸಂಶೋಧನೆಗಳಲ್ಲಿ ನಿಮ್ಮ ಮಗುವನ್ನು ಒಳಗೊಂಡಿರುವಂತೆ ನಿಮ್ಮನ್ನು ಕೇಳಲಾಗಿದೆ.

ಪರಿಚಯ

ಅಧ್ಯಯನದ ಉದ್ದೇಶ:

ನಿಮ್ಮ ಮಗುವಿನ ಭಾಗವಹಿಸುವಿಕೆ ಪಿ ಡಿ ಎಚ್ ಹೆಚ್ ಎಂ ಮತ್ತು ಡಿಬಾಚ್ಚ್‌ನಲ್ಲಿನ ಇತರ ವೀಕ್ಷಣೆಯ ಫರಿತಾಂಶಗಳನ್ನು ಪಡೆಯುವ ಮೂಲಕ ನಿರ್ದೇಶನಗಳಲ್ಲಿ ಎಂಟ್ರಿ ಕೊಡಬೇಕು ಸಂಭವನೀಯತೆಯನ್ನು ತಿಳಿಯಲು ನಮಗೆ ಸಹಾಯ ಮಾಡುತ್ತದೆ. ಯಾವುದೇ ಕಾರಣಕ್ಕಾಗಿ ಯಾವುದೇ ಸಮಯದಲ್ಲಿ ನೀವು ಪಾಲ್ಗೊಳ್ಳುವಿಕೆಯನ್ನು ಭಾಗವಹಿಸುವುದನ್ನು ನಿಲ್ಲಿಸಲು ಮತ್ತು ಸಂಶೋಧನೆಯಲ್ಲಿ ಪಾಲ್ಗೊಳ್ಳಲು ನಿಮಗೆ ಯಾವುದೇ ಮರುದಾವತಿಯನ್ನು ನೀಡಲಾಗುವುದಿಲ್ಲ. ಆದ್ದರಿಂದ ನಿಮ್ಮ ಮಗುವನ್ನು ಅಧ್ಯಯನದಲ್ಲಿ ಕೊಡಗಿಸುವುದು ನಿಮ್ಮ ಸ್ವಯಂಪ್ರೇರಿತ ನಿರ್ಧಾರ.

ಸ್ವಯಂಪ್ರೇರಿತ ಭಾಗವಹಿಸುವಿಕೆ

ಈ ಅಧ್ಯಯನದ ನಿಮ್ಮ ಮಗುವಿನ ಪಾಲ್ಗೊಳ್ಳುವಿಕೆ ನಿಮ್ಮ ಸ್ವಯಂಪ್ರೇರಿತ ನಿರ್ಧಾರವಾಗಿದೆ, ಭಾಗವಹಿಸಬೇಕೇ ಅಥವಾ ಇಲ್ಲವೇ ಎಂಬುದನ್ನು ನಿಮ್ಮ ಪ್ರಸಕ್ತ ಅಥವಾ ಭವಿಷ್ಯದ ಸಂಬಂಧ ಕೆಎಲ್‌ಎಎಸ್ ಡಾ. ಪ್ರಬಾಕರ್ ಕೋಲೆ ಆಸ್ಪತ್ರೆ ಮತ್ತು ಎನ್‌ಎಸ್‌ಫಿ, ಬೆಳಗಾವಿ ಗಳೊಂದಿಗೆ ಪರಿಶಾಮ ಬೀರುವುದಿಲ್ಲ.

ಅಪಾಯ ಮತ್ತು ಪ್ರಯೋಜನಗಳು

ಒಳಗೊಂಡಿರುವ ಯಾವುದೇ ಅಪಾಯಗಳಿಲ್ಲ.

ರೋಗ ಹರಮವಿಕ ಮತ್ತು ಮರಣ ಪ್ರಯೋಜನದಲ್ಲಿ

ಗೌಪ್ಯತೆ ಮತ್ತು ಗೌಪ್ಯತೆ

ನೀವು ಸಂಶೋಧನಾ ಪಾಲ್ಗೊಳ್ಳುವವರು ಎಂದು ಸಂಶೋಧಿಸುವವರು ಮಾತ್ರ ಸಂಶೋಧನಾ ತಂಡದ ಸದಸ್ಯರಾಗಿದ್ದಾರೆ. ನಿಮ್ಮ ಬಗ್ಗೆ ಅಥವಾ ನೀವು ಒಡಗೂಡಿದ ಯಾವುದೇ ಮಾಹಿತಿಯಿಲ್ಲದೆ, ಸಂಶೋಧನೆಯ ಸಮಯದಲ್ಲಿ ನಿಮ್ಮ ರಿಖಿತ ಅನುಮತಿಯಿಲ್ಲದೆ ಇತರರಿಗೆ ಒಹಿರಂಗಪಡಿಸಲಾಗುತ್ತದೆ ಸಮೂಹಗಳಲ್ಲಿ ಸಂಶೋಧನೆಯ ಫರಿತಾಂಶಗಳನ್ನು ಪ್ರಕಟಿಸಿದಾಗ ಅಥವಾ ಚರ್ಚಿಸಿದಾಗ, ನಿಮ್ಮ ಗುರುತನ್ನು ಒಹಿರಂಗಪಡಿಸುವ ಯಾವುದೇ ಮಾಹಿತಿಯನ್ನು ಒಹಿರಂಗಪಡಿಸಲಾಗುವುದಿಲ್ಲ. ಈ ಅಧ್ಯಯನದೊಂದಿಗೆ ಸಂವರ್ಕ ಹೊಂದಿದ

ಮತ್ತು ನಿಮ್ಮೊಂದಿಗೆ ಗುರುತಿಸಬಹುದಾದ ಯಾವುದೇ ಮಾಹಿತಿ ಗೌಪ್ಯವಾಗಿ ಉಳಿಯುತ್ತದೆ ಮತ್ತು ನಿಮ್ಮ ಅನುಮತಿಯೊಂದಿಗೆ ಮಾತ್ರ ಪ್ರಕಟಗೊಳ್ಳುತ್ತದೆ.

— — — — —

ದೂರವಾಣಿ ಸಂಖ್ಯೆ 9945301283

ದೂರವಾಣಿ _____ ಎಕ್ಸಾಮ್ ಸಂಖ್ಯೆ

ನಿಮ್ಮ ಹಕ್ಕುಗಳು ಅಥವಾ ಸಂಪೋಷನೆಯ ಭಾಗವಹಿಸುವಿಕೆ ಬಗ್ಗೆ ನೀವು ಯಾವುದೇ ಪ್ರಶ್ನೆಗಳನ್ನು ಹೊಂದಿದ್ದರೆ, ನೀವು ಸಂಪರ್ಕಿಸಬಹುದು

ಆದ್ಯಕ್ಷ ನೈತಿಕ ಸಮಿತಿ:

ಡಾ. ದೂಪಾ ಎಂ ಬೆಲ್ಟಾಡ್ ಎಮ್ ಡಿ ಡಿ ಸಿ ಸಿ ಹೆಚ್
ಪ್ರೊಫೆಸರ್
ಪೀಡಿಯಾಟ್ರಿಕ್ಸ್, ಉಲಾಪ್,
ಕಾಕರ್ ಜವಾಹರ್‌ಲಾಲ್
ನಹರು ಮೆಡಿಕಲ್ ಕಾಲೇಜ್,
ಲೆಳಗಾವಿ - 590010

ನಿಮ್ಮ ಮಾಹಿತಿಗಾಗಿ ಈ ಫಾರ್ಮ್ ಸಕಲವು ನಿಮಗೆ ನೀಡಲಾಗುವುದು ಮತ್ತು ನಿಮ್ಮ ದಾಖಲೆಗಳಿಗಾಗಿ ಇರಿಸಿಕೊಳ್ಳಬಹುದು.

अनुसंधान में भागीदारी के लिए सहमति

"प्रोस्टिम नियोनेट्स के बीच एनईसी की घटनाएं पाश्चराइज्ड दाता मानव दूध प्राप्त करने के लिए केएलई डॉ प्रभाकर कोरे अस्पताल और एमआरसी, बेलगावी में एक साल का पार अनुभागीय अध्ययन।"

सहभागिता: डा. सौरभ, पीजी छात्र

आपको जवाहरलाल नेहरू मेडिकल कॉलेज, बेलगावी में पेडियाट्रिक्स विभाग में पीजी छात्र डॉ. सौरभ द्वारा काहेर जेएन मेडिकल कॉलेज अस्पताल, बेलगावी के एनआईसीयू में आयोजित उपरोक्त शोध में अपने बच्चे को शामिल करने के लिए कहा गया है।

परिचय

अध्ययन का उद्देश्य:

आपके बच्चे की भागीदारी से हमें पीडीएचएम प्राप्त करने वाले पूर्ववर्ती नियोनेट्स और निर्वहन पर अन्य अवलोकन परिणामों में नेक्रोटाइजिंग एंटेरोकोलिसिस की घटनाओं को जानने में मदद मिलेगी। आप किसी भी समय किसी भी कारण से अध्ययन में भागीदारी को बंद करने के लिए स्वतंत्र हैं और आपको शोध में भागीदारी के लिए कोई प्रतिपूर्ति नहीं दी जाएगी। इसलिए अध्ययन में अपने बच्चे को शामिल करना आपका स्वैच्छिक निर्णय है।

स्वैच्छिक भागीदारी

इस अध्ययन में आपके बच्चे की भागीदारी आपका स्वैच्छिक निर्णय है, चाहे भाग लेना चाहे या नहीं, केएलई डॉ प्रभाकर कोरे अस्पताल और एमआरसी, बेलगावी के साथ आपके वर्तमान या भविष्य के संबंधों को प्रभावित नहीं करेगा।

जोखिम और लाभ

इसमें कोई जोखिम शामिल नहीं है।

विकृति और मृत्यु दर में कमी।

गोपनीयता और गोपनीयता

एकमात्र लोग जो यह जान लेंगे कि आप एक शोध भागीदार हैं, वे शोध दल के सदस्य हैं। आपके बारे में कोई जानकारी या आपके द्वारा प्रदान की गई, अनुसंधान के दौरान दूसरों को आपकी लिखित सहमति के बिना खुलासा किया जाएगा। जब शोध के परिणाम सम्मेलनों में प्रकाशित या चर्चा की जाती हैं, तो कोई जानकारी प्रकट नहीं की जाएगी जो आपकी पहचान प्रकट करेगी। इस अध्ययन के संबंध में प्राप्त की गई कोई भी

जानकारी और आपके साथ पहचाना जा सकता है। गोपनीय रहे और केवल आपकी अनुमति के साथ ही खुलासा किया जाएगा।

यदि आपके अधिबन्धों या शोध भागीदारी के बारे में आपके कोई प्रश्न हैं तो आप संपर्क कर सकते हैं।

अध्यक्ष नैतिक समिति:

डॉ. रूपा एम. बेलाद, एमडी, डीसीएच

प्रोफेसर

पेट्रियाट्रिक्स विभाग,

काहेर जेएन रोडिकल कॉलेज,

बेतगावी 590010

आपको अपनी जानकारी के लिए और अपने रिकॉर्ड रखने के लिए इस फॉर्म की एक प्रति दी जाएगी।

ANNEXRURE - II

PROFORMA/DATA COLLECTION INSTRUMENT

**INCIDENCE OF NEC AMONG PRETERM NEONATES RECEIVING
PASTEURISED DONOR HUMAN MILK – A CROSS SECTIONAL STUDY AT
KLE DR. PRABHAKAR KORE HOSPITAL AND MRC, BELAGAVI**

I. DEMOGRAPHIC EXAMINATION

1. Identification Number:
2. In patient Number:
3. Date of admission:
4. Name:
5. Religion: Hindu Muslim Sikh Others
6. Address:
7. Age: hours of life
8. Gender Male Female Ambiguous
9. Mothers Name
10. Mother's education: Illiterate Primary Secondary Graduate
Post Graduate
11. Mother's occupation: Home maker Employed Self
employed

12. Father's Name:

13. Father's education: Illiterate Primary Secondary Graduate
Post Graduate

14. Number of members at home:

15. Income

16. Per capita

17. Socio economic status according to the Modified B. G. Prasad's Classification

- a. Upper class (<Rs. 5775)
- b. Upper middle class (Rs 2887 to 5774)
- c. Middle class (Rs. 1733 to 2886)
- d. Lower middle class (Rs. 866 to 1732)
- e. Lower class (<Rs. 866)

18. Death of siblings: Yes No

19. Birth order of child:

20. Family history: Consanguineous Non-Consanguineous

21. General information given by: Mother Father

22. Written informed consent Yes No

II Maternal history:

a. Maternal age: 18-20 21-25 26-30 31-35 5

b. Gravida: Primi Gravida 2 Gravida 3
Multi

c. Antenatal visits Not done < 3 >3

d. Antenatal USG scans Done Not done

e. Remarks

23. Antenatal risk factors for preterm birth

- | | |
|----------------------------------|--------|
| a. Elderly primi > 35 years | Yes/No |
| b. Short statured < 145 cm | Yes/No |
| c. Preeclampsia and eclampsia | Yes/No |
| d. Anaemia | Yes/No |
| e. Gestational diabetes mellitus | Yes/No |
| f. Previous stillbirth, IUD | Yes/No |
| g. Previous caesarean section | Yes/No |
| h. Grand multipara | Yes/N |
| i. PPRM | Yes/No |
| j. Non reactive NST | Yes/No |
| k. Twins | Yes/No |

III. Birth history

1. Mode of Delivery: NVD LSCS Instrumental
delivery
2. Gestational age in weeks: < 28 28 to 34 > 34
3. Gestational age by modified ballard score: weeks
4. APGAR score at five minutes < 7 7

IV Indication of NICU Admission

1. Indication

- | | |
|----------------------------------|--------|
| a. Low birth weight | Yes/No |
| b. Respiratory distress syndrome | Yes/No |
| c. MAS | Yes/No |
| d. Hyperbilirubinemia | Yes/No |

- e. Feeding difficulties Yes/No
- f. Observation Yes/No
- g. Hypoglycemia Yes/No
- h. Any other Yes/No

2. Examination

a. General physical examination

- i. Heart rate per minute
- ii. Respiratory rate per minute
- iii. CFT seconds
- iv. Temperature °F

3. Anthropometry

Parameters	Measured	Expected
Weight (Kg)		
Length (Cm)		
Head circumference (Cm)		
Abdominal girth (Cm)		

4. Head to toe examination

- a. Face
- b. Eyes
- c. Ears
- d. Oral cavity
- e. Neck
- f. Chest
- g. Chest

- h. Abdomen
 - i. Extremities
 - j. Congenital markers
 - k. Skin
5. Other systems
- a. Cardiovascular system
 - b. Respiratory system
 - c. Per abdomen
 - d. Central nervous system
6. Feeding history

Type	Day of life																											
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
MDHM																												
PDHM																												
Formula																												
Amount																												
Method																												
BF																												
S.F. Feeding																												
Direct																												

7. Total duration of PDHM given Days and
Hours

8. Initiation of breast feeding:

9. Use of probiotics Yes/No
 If yes, Total duration of probiotic administration

10. Acceptability of PDHM Yes/No
 If No, Reasons

a. Parent refusal to accept Yes/No

- b. Grand parents did not allowing parents to accept PDHM Yes/No
- c. Fear that PDHM may cause any disease to the baby Yes/No
- d. Social factors (Cast, creed) Yes/No
- e. Any other Yes/No

11. Investigations

Haemoglobin (gm%)							
Platelet count (/cumm)							
WBC (/cumm)							

Hs-CRP

Blood culture

Sodium levels

ABG

Radiograph

Stool for occult blood

Ultrasound

12. Any Transfusions

- a. RDP Yes/No
- b. FFP Yes/No
- c. PCV Yes/No

13. Development of NEC Yes/No

14. If yes Stage Stage 1 Stage 2 Stage 3

15. Mortality

16. If Yes, Reason

- | | |
|----------------------------|--------|
| a. Preterm | Yes/No |
| b. LBW/VLBW/ELBW | Yes/No |
| c. Sepsis | Yes/No |
| d. DIC | Yes/No |
| e. Ill defined/unspecified | Yes/No |

17. Course in the hospital

18. Stay in days

- | | | |
|---------------|---|------|
| a. NICU | <input type="text"/> <input type="text"/> | Days |
| b. NICU+KMC | <input type="text"/> <input type="text"/> | Days |
| c. KMC | <input type="text"/> <input type="text"/> | Days |
| d. Post natal | <input type="text"/> <input type="text"/> | Days |

19. Anthropometry at discharge

- | | | |
|-----------------------|---|-------|
| a. Weight | <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> | grams |
| b. Length | <input type="text"/> <input type="text"/> | cms |
| c. Head circumference | <input type="text"/> <input type="text"/> | cms |

20. Types of feeds at discharge

- | | |
|---------------------------|--------|
| a. Breast feed | Yes/No |
| b. If Yes, | |
| i. Direct breast feed | Yes/No |
| ii. Expressed breast milk | Yes/No |
| iii. Both | Yes/No |

21. PDHM Yes/No

- | | | |
|------------------|--|--------|
| a. If yes amount | <input type="text"/> <input type="text"/> <input type="text"/> | mL/day |
|------------------|--|--------|

22. Formula Yes/No

- | | |
|-------------------|--|
| a. If yes, amount | |
| i. Is Yes Amount | <input type="text"/> <input type="text"/> <input type="text"/> day |

ANNEXURE-III-ETHICAL CLEARANCE LETTER



K.L.E. ACADEMY OF HIGHER EDUCATION AND RESEARCH
(Deemed - to be University)

Accredited 'A' Grade by NAAC (2nd Cycle)

Placed in Category 'A' by MHRD (GoI)

JAWAHARLAL NEHRU MEDICAL COLLEGE,
NEHRU NAGAR, BELAGAVI-590010 (KARNATAKA-INDIA)

Website: <http://www.jnmc.edu>
E-Mail : dome@jnmc.edu

Phone: (+91 (0)831 Office : 2472550
Principal : 2471701
Fax No. +91 (0)831 - 2470759

Ref: MDC/DOME/ 69

Date: 24/11/2018

To,

(REG

NO.BM0118005)

Sub: Institutional Ethical Clearance for the study.

With reference to the above, we wish to inform you that your proposed research project titled "INCIDENCE OF NECROTIZING ENTEROCOLITIS AMONG PRETERM NEONATES RECEIVING PASTEURIZED DONOR HUMAN MILK-A ONE YEAR CROSS SECTIONAL STUDY AT KLE DR. PRABHAKAR KORE HOSPITAL AND MRC, BELAGAVI", is ethical and justifiable. The proposed research project has been cleared by the JNMC Institutional Ethics Committee on Human Subjects Research.

(Dr. Arathi Darshan)
Member Secretary
JNMC Institutional Ethics Committee
on Human Subjects Research,
J.N.Medical College, Belagavi.

(Dr. Roopa M Bellad)
Chairman,
JNMC Institutional Ethics Committee
on Human Subjects Research,
J.N.Medical College, Belagavi.

ANNEXURE-V**KEY TO MASTER CHART**

ABG	-	Arterial blood gas
Acceptability of PDHM		
N	-	No
Y	-	Yes
Antenatal risk factors for preterm birth		
1	-	Elderly primi > 35 years
2	-	Short statured < 145 cm
3	-	Preeclampsia and eclampsia
4	-	Anemia
5	-	Gestational diabetes mellitus
6	-	Previous still birth, intra uterine death
7	-	Previous caesarean section
8	-	Grand multipara
9	-	Preterm premature rupture of membranes
10	-	Non reactive Nonstress test
11	-	Twins
Antenatal USG scans		
N	-	Not done
Y	-	Done
Antenatal visits		
1	-	Not done
2	-	< 3
3	-	> 3

Birth order

- | | | |
|---|---|--------|
| 1 | - | First |
| 2 | - | Second |
| 3 | - | Third |

Blood culture

- | | | |
|-----|---|-------------------------|
| AB | - | Acinetobacter baumannii |
| AW | - | Acinetobacter wolffii |
| BC | - | Burkholderia cepacia |
| CA | - | Candida albicans |
| CG | - | Candida glabrata |
| CK | - | Candida kruzi |
| CT | - | Candida tropicalis |
| EF | - | Entrococcus faecium |
| ENT | - | Enterococcus |
| KP | - | Kleibsella pneumoniae |
| NG | - | No growth |

Consanguineous marriage

- | | | |
|---|---|-----------------------------|
| 1 | - | Consanguineous marriage |
| 2 | - | Non consanguineous marriage |

Death of sibling

- | | | |
|---|---|-----|
| N | - | No |
| Y | - | Yes |

DL (on radiograph)	-	Distended bowel loops
--------------------	---	-----------------------

Fathers' education

1	-	Illiterate
2	-	Primary
3	-	Secondary
4	-	Graduate
5	-	Post graduate

Feeding history

MOM	-	Mother's own milk
PDHM	-	Pasteurized donor human milk
RTF	-	Ryles tube feeding
S/F/Paladai	-	Spoon feed

Gestational age in weeks

1	-	< 28
2	-	28 to 34
3	-	> 34

Gender

A	-	Ambiguous
F	-	Female
M	-	Male

Gravida

1	-	Primi gravida
2	-	Gravida 2
3	-	Gravida 3
4	-	Multi

Indication of NICU admission

- 1 - Low birth weight
- 2 - Respiratory distress syndrome
- 3 - Meconium aspiration syndrome
- 4 - Hyperbilirubinemia
- 5 - Feeding difficulties
- 6 - Observation
- 7 - Hypoglycemia
- 8 - Any other / Kangaroo mother care

MA - Metabolic acidosis

Maternal age in years

- 1 - 18 to 20
- 2 - 21 to 25
- 3 - 26 to 30
- 4 - 31 to 35
- 5 - > 35

Mode of delivery

- 1 - Normal vaginal delivery
- 2 - Lower segment caesarean section
- 3 - Instrumental delivery

Mothers' education

- 1 - Illiterate
- 2 - Primary
- 3 - Secondary
- 4 - Graduate

5	-	Post graduate
N	-	No
Occupation		
1	-	Homemaker
2	-	Employed
3	-	Self employed
PDHM	-	Pasteurized donor human milk
Platelet count		
K	-	Thousand
L	-	Lacs
Religion		
1	-	Hindu
2	-	Muslim
3	-	Sikh
4	-	Others
Socio economic status according to modified BG Prasad's classification		
1	-	Upper class
2	-	Upper middle class
3	-	Middle class
4	-	Lower middle class
5	-	Lower class
Stage of NEC		
1	-	Stage 1
2	-	Stage 2
3	-	Stage 3

Type of feed at discharge

- B - Breastfeed
- P - Pasteurized donor human milk

Transfusion

- 1 - RDP (Randomized donor pletelat)
- 2 - FFP (Fresh frozen planes)
- 3 - PCV (Packed cell volume)

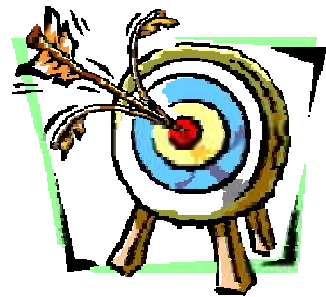
USG - Ultrasonography

White blood cell

- K - Thousand
- L - Lacs



Introduction



Objectives



Review of Literature



Methodology



Results



Discussion



Limitation



Conclusion



Summary



Bibliography



Annexure-I



Annexure-II



Annexure-III



Annexure-IV



Annexure-V
