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Probiotics in Prevention of NEC in Preterms Babies

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Introduction

Necrotizing Enterocolitis **(**NEC**)** is an acute intestinal necrosis syndrome of unknown etiology. Its pathogenesis is complex and multifactorial. **(**1**)**

Necrotizing Enterocolitis is an inflammatory bowel disease of neonates and remains one of the most common gastrointestinal emergencies in new born infants. Onset of NEC is often within the first three months of life and the neonates who are of extremely low birth weight **(**<1000gms**)** and under 28weeks gestation are the most susceptible. Full term neonates account for 10% cases of all NEC while premature account for 90%. **(**2**)**

The incidence of NEC varies from centre to centre and from year to year within centers. An estimated 0.3 to 2.4 cases occur in every 1000 live births. In most centers, NEC occurs in 2-5% of all NICU admissions and 5-10% of very low birth weight. If VLBW infants who die very early are excluded and only infants who have been fed included, the incidence is approximately 15%. **(**1**)**

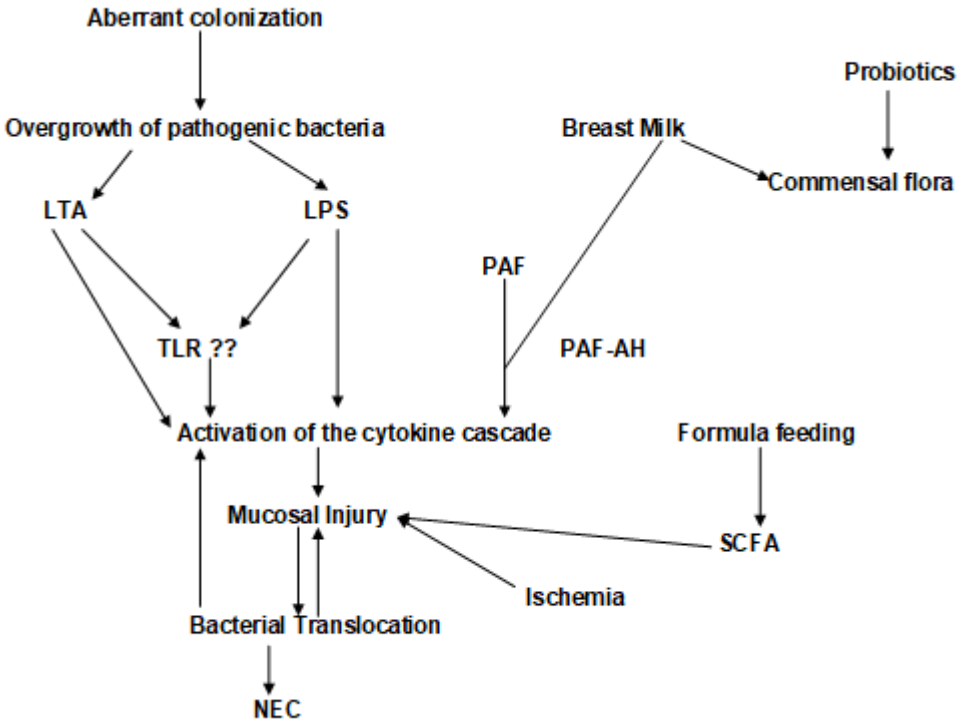
Prematurity is the single greatest risk factor. Decreasing gestational age is associated with increased risk of NEC. The mean gestational age for NEC is 30-32 weeks, and the infants are generally weight appropriate for gestational age. The overall mortality is 9%-28% regardless of medical or surgical intervention. The mortality for infants weighing <1500 grams can be as high has 45%, for those weighing <750 grams, it may be much higher. **(**1**)**

Pathogenesis

Risk factors associated with NEC have been suggested as prematurity ,infectious- pathogenic bacteria/viral colonization of lumen, sepsis, oxygen delivery - consumption imbalance **(**perinatal hypoxia and ischemia, congenital heart disease, anemia, abnormal hemoglobins, polycythemia**)**, and iatrogenic **(**umbilical arterial or venous catheterization; drugs - indomethacin, methylxanthines, H2 blockers; feeding regimens - advancing too fast, high osmolality; feeding additives - calcium, vitamin E; and formula feeding**)**.

Pathogenesis of NEC is poorly understood. It may be a multifactorial disorder: prematurity, enteral feeding and uncontrolled inflammation in the bowel are three important factors for development of NEC **(**8**)**

Flow diagram of proposed factors involved in the pathogenesis of NEC. LTA – Lipoteichoic acid; LPS– Lipopolysaccharide; TLR – Toll like receptor; PAF – platelet activating factor; PAF-AH - platelet activating factor acetyl-hydrolase; SCFA – Short chain fatty acids; NEC – Necrotizing enterocolitis. (8**)**



Clinical Findings

A change in feeding tolerance with gastric retention is a frequent early sign. Vomiting, feeding intolerance, abdominal distension and periumbilical and flank erythema on the abdominal wall, blood in the stools, lethargy, apnea, and temperature instability; and in severe cases, progressive systemic shock with metabolic acidosis, oliguria, hypotension and disseminated intravascular coagulation **(**DIC**)** may develop.

When NEC is suspected, serial abdominal X-ray films are recommended to check for the presence of pneumatosis intestinalis and pneumoperitoneum and for assessing disease progression. Occult blood in stool and sepsis are evaluated in suspected cases. The presence of abdominal distention, blood in stool, and pneumatosis intestinals confirm the clinical diagnosis of NEC. However, presence of occult blood is not specific for NEC. At least one positive occult blood is found in 58% of infants <1800 g over a six-week period **(**3**)**.

Pneumatosis intestinalis **(**gas bubbles within the bowel walls**)** is thought to be produced by bacterial fermentation of substrates and diagnostic of NEC **(**present in 85% of cases**)**. In severe cases, portal air can be seen and is associated with severe bowel necrosis in about 40% of the cases. However, radiological signs may vary with gestational age; pneumatosis intestinalis is present in 100% of full-term infants and in 29% of infants whose gestational ages are ≤26 weeks, while portal venous gas is present in 47% and 10%, respectively **(**4**)**. Pneumoperitoneum is present in severe cases. "Football sign" for free gas in the peritoneal cavity is a large hypolucent area in the central abdomen with markings from the falciparum ligament. Pneumatosis coli **(**pneumatosis in the colon without small intestinal involvement**)** is a benign form of NEC. Sonographic findings are also useful in predicting outcome and therefore might help guide management **(**5**)**.

The severity of the disease was categorized in stages by Bell et al. **(**6**)** in 1978, later modified by Walsh and Kliegman **(**7**)** in 1986. Briefly, abdominal distention in stage I **(**mild**)**, pneumatosis intestinalis in stage II **(**moderate**)**, and pneumoperitoneum in stage III **(**severe**)** are the diagnostic parameters. In 25% of cases, NEC is suspected but not confirmed **(**stage I**)**. The symptoms resolve gradually in these infants. In 25-40% of cases, the progression of NEC is fulminant with sepsis, DIC, and shock **(**stage III**)**.

Predominant pathological lesion is coagulative or ischemic necrosis and most commonly involves the ileocecal region **(**insufficient blood supply?**)**. In about half of the cases, the necrosis involves both the small and large intestines, either continuous or segmental. In severe cases, gas bubbles, which may be grossly visible in the intestinal wall, involve the entire colon more commonly in the term infant than in the premature.

Bells Staging Criteria for NEC (2)

Stage	*Systemic signs*	*Intestinal signs*	*Radiological signs*
I A (Susp)	Temperature instability, bradycardia, Lethargy, Apnea.	Poor feeding, emesis, Incr -ease pregavage residuals Mild abdominal distension	Normal or intestinal dilatation, mild illeus
I B (Susp)	Same as above	Above+Blood from Rectum	Same as above
IIA (Proven)	Same as above	Above+absent bowel sounds +mild abdominal tenderness	Intestinal dilatation, Illeus,Pneumatosis Intestinalis.
IIB (Proven)	Above + Metabolic Acidosis+Thromboc -topenia	Above+Definite abdominal tenderness	Above + Portal vein gas+possible ascities
IIIA (Advanced)	Above+Hypotension +respiratory acidosis	Above+Peritonitis,marked distension of abdomen	Above+definiteascities
IIIB (Advanced)	Same as above	Same as above	Above+Pneumoperitoneum

Laboratory features:

The diagnosis is suspected from the clinical presentation but must be confirmed by diagnostic radiographs, surgery, or autopsy. No laboratory tests are specific for NEC; nevertheless, some tests are valuable in confirming the diagnostic impressions.

Radiologic studies:

The abdominal radiograph will often reveal an abnormal gas pattern consistent with illeus. Both anterior posterior and cross table lateral or left lateral decubitus views should be included. These films may reveal bowel wall edema, a fixed position loop on serial studies, the appearance of a mass, pneumatosis intestinalis **(**the radiological hallmark used to confirm the diagnosis**)**, portal or hepatic venous air, pneumobilia, or pneumoperitoneum. Isolated intestinal perforation may present with pneumoperitoneum without other Clinical signs (1)

Blood studies:

Thrombocytopenia, persistent metabolic acidosis, and sever refractory hyponatremia constitute the most common triad of signs and help to confirm the diagnosis. Serial measurements of CRP may also be helpful in the diagnosis and assessment of response to therapy of severe NEC.**(**1**)**

Analysis of Stool for blood and carbohydrate has been used to detect in infants with NEC based on changes in intestinal integrity. Although grossly bloody stools may be an indication of NEC, occult hematochezia does not correlate well with NEC.

Carbohydrate malabsorption, as reflected in a positive stool Clinitest result, can be frequent and early indicator of NEC within the setting of signs noted in clinical characteristics. **(**1**)**

Management (1)

The mainstay of treatment remains medical stabilization.

Stage I NEC – NPO with IV fluids, Nasogastric drainage, CBC, electrolytes, KUB q 6-8hrly for 48 hrs, blood culture, stool for occult blood. Ampicillin and gentamycin for 48 hours.

Stage 2 NEC – NPO with parenteral nutrition **(**by CVL once sepsis is ruled out**)**, Nasogastric drainage, CBC, Electrolytes, KUB **(**AP and lateral**)** q 6-8 hrly for 48 to 72 hrs, then prn, blood culture, stool for occult blood. Ampicillin, gentamycin and clindamycin for 14 days and surgical consultation.

Stage 3 NEC - NPO with parenteral nutrition **(**by CVL once sepsis is ruled out**)**, Nasogastric drainage, CBC, Electrolytes, KUB **(**AP and lateral**)** q 6-8 hrly for 48 to 72 hrs, then prn, blood culture, stool for occult blood. Ampicillin, gentamycin and clindamycin for 14 days and surgical consultation with intervention , if indicated: Resection with enterostomy or primary anastomosis. In selected cases **(**usually < 1000grams and unstable**)**, bedside drainage under local anaesthesia.

Prevention

Stratergies to prevent NEC (2**)**

Evidence-based support for efficacy	**Limited data to support efficacy**
Breast feeding	Cautious advancement of feeding
Trophic feeding	Fluid restriction
Antenatal steroids	Oral immunoglobulins
Enteral administration of antibiotics	L-arginine supplementation
	Polyunsaturated fatty acids
	Acidification of milk feeds
	Probiotics, prebiotics & postbiotics
	Growth factors & Erythropoietin
	Free radical scavengers

Human milk- has been reported to reduce the incidence of NEC by upto 10 fold compared with infant formula whether using mothers own or donor milk. The protective effect of breast milk has been correlated with its anti-inflammatory components **(**IL10**)**, growth factors, erythropoietin, lysozyme, immunoglobulins as well as probiotics that modulate intestinal microflora composition to the advantage of the host. The activity of acetyl hydrolase **(**PAF-AH**)**, an enzyme that degrades PAF, is lower in neonates less than 3 weeks of age than any other time. The additional presence of PAF-AH activity may also partly explain the protective effect of breast milk, as infant formulas do not contain it. **(**2**)**

Trophic feeds- Initiation of trophic feeds, small volumes of breast milk or formula, may overcome gut atrophy and inflammatory responses associated with prolonged bowel rest. Trophic feeds improve the activity of digestive enzymes, enhance the release of digestive hormones and increase intestinal blood flow and digestive motility in premature infants.

Antenatal glucocorticoids have been reported to alter immune system development in very premature infants. Antenatal glucocorticoid therapy has beneficial effects by suppressing inflammation and promoting gastrointestinal maturation and function including reduced mucosal uptake of macromolecules, decreased colonization with aerobic bacteria, and increased activity of enzymes such as lactase, maltase, sucrase and Na/K ATP ase.

Standardized feeding regimens- A significant decline 87% of incidence of NEC and 29% in the risk of developing NEC was reported following implementation of standardized feeding regime.

A Systematic review and meta analyses indicates that resticted water intake significantly increases postnatal weight loss and significantly reduces the risk of NEC.

Probiotics

One of the stratergies to prevent NEC are probiotics. Many studies have been done on probiotics in preventing NEC in preterm babies.

The term probiotic was introduced into the scientific literature in 1965 by Stillwell and Lilly. Probiotic bacteria are defined as live microbial supplements that colonise the gut and provide benefit to the host. **(**9**)**The most frequently used genera fulfilling these criteria are lactobacillus and bifidobacterium. **(**10**)**

The concept that the bacteria live within us may be important determinants of health and disease was proposed by Metchnikov and popularized by Douglas. **(**9**)**

Often credited as the first advocate for probiotics, Elie Metchnikoff, the father of immunology, investigated intestinal microbes as causative agents in aging, a process he called “autointoxication”. He made the observation that lactic fermentation of milk products arrested putrefaction and suggested that the consumption of those products might offer the same protection to human.

For the organisms to be considered as probiotics, the following criteria need to be fulfilled: **(**10**)**

- It should be isolated from the same species as its intended host.
- It should have a demonstratable beneficial effect on the host.
- It should be non-pathogenic.
- It should be able to survive transit through the gastrointestinal tract.
- On storage large number of viable bacteria must be able to survive prolonged periods.

The microbiota of a newborn develops rapidly after the birth and it is initially dependent mainly on the mother's microbiota, mode of delivery, birth envoirnment and rarely genetic factors. The maternal vaginal and intestinal flora constitutes the source of bacteria, which colonizes the intestine of the newborn, the dominating strains being facultative anaerobes such as the enterobacteria, coliforms, and lactobacilli. After weaning the composition of the microflora gradually alters to resemble that of the adult. The bacterial strains with beneficial properties include mainly bifidobacteria and lactobacilli. **(**10**)**

The fecal flora of 46 preterm infants and 52 born at full term was studied at 10 days of age; 46 born at full term and 37 preterm infants were also studied at 30 days. Gas liquid chromatography was used to identify the anaerobes lactobacilli, but not bifidobacteria, were found in high counts in the stools of most of infants born at full term by 30 days of age. The mode of delivery, but not the method of feeding, had a significant influence on early colonization. A selective deficiency of lactobacilli compared with coliform organism was found in preterm infants. There study indicated that lactobacilli may be an important part of normal stool flora in early infancy, and that modern methods of neonatal care are associated with delayed or deficient colonization. **(**11**)**

A high proportion of preterm infants receiving intensive care suffer episodes of systemic infection with antibiotic resistant bacteria and fungi **(**12**)**. These infections further increase the risk of adverse outcomes such as chronic lung disease and brain injury **(**12, 13**)**.

There are several mechanisms by which probiotic administration may be expected to decrease the incidence of infection in preterm infants.

- Changes in intestinal permeability.
- Enhanced mucosal Ig A responses.
- An increase in production of anti-inflammatory cytokines.
- An increase barrier to translocation of bacteria and bacterial products across mucosa.**(**14, 15**)**
- Changes in the pattern of gastro intestinal tract colonization, leading to a decrease in the extent to which preterm infants are colonized with potential pathogens such as enterococci **(**16**)** and possibly increase colonization with desirable microflora such as streptococcus salivarius **(**17**)**

Potential use of probiotics could lead to improvements in nutrition, reduced dependence on intravenous nutrition, a reduction in the incidence of sepsis and use of antibiotics and prevention of NEC.**(**9**)**

Normalization of the properties of unbalanced indigenous microflora by specific strains of the healthy gut microflora constitutes the rationale of probiotic therapy. **(**10**)**

Modification of the intestinal flora by increasing the predominance of specific nonpathogenic bacteria seemed to be a reasonable means of attaining a prophylactic or therapeutic affect against enteropathogens. **(**10**)**

Selection of strains for clinical trails is based on the microbial characteristics such as ability to survive gastric acid and colonize the gut, products of factors that inhibit the growth of pathogenic bacteria **(**such as H2O2 by lactobacilli**)** and other desirable **(**generally metabolic or immunologic effects**)**. **(**9**)**

The microorganism most frequently used as probiotic agents are lactic acid bacteria **(**species of lactobacillus**)** and nonpathogenic, antibiotic resistant, ascosporic yeasts, such as saccharomyces boulardi. Lactobacillus rhamnosus GG, which was originally isolated from human intestinal flora, is the most widely studied probiotic agent for adults and children **(**18**)**

The probiotic organism that has received the most clinical attention to date is known as Lactobacillus rhamnosus strain GG. The isolation in 1985 of LGG began with the realization that lactobacillus strains traditionally used for fermentation by the dairy industry were unable to implant the human gut. LGG was discovered by developing a list of ideal qualities for a lactobacillus strain which include attachment to intestinal cells, colonization in human intestinal tract,resistance to acid and bile, produces antimicrobial substance and beneficial effects on human health, and then searching in the microflora of healthy humans for a naturally occurring lactobacillus strain that possessed the necessary characteristics. Thus, LGG was not manipulated, mutated, or altered in any way;it was found by natural selection. An additional benefit of LGG is its unique colonial morphology, which makes it easy to identify in a mixed culture of other lactobacilli and streptococci, such as those encountered in fecal cultures. **(**18**)**

Trails showing decrease of NEC in population of premature newborns given supplements of lactobacillus GG daily compared to historical control subjects have been reported. **(**19,20**)**. These findings suggested a correlation between the reduction of lactobacilli and the risk of NEC.

An important rationale for the use of probiotics in neonates at risk for NEC is the observation that very low birth weight infants have aberrant fecal colonization compared with healthy term infants. The predominant facultative species in the fecal flora of preterm infants undergoing intensive care are staphylococci **(**CONS and staphylococcus aureus**)**, enterobacteriaceae **(**klebsiella**)** and enterococci. Clostridia are the most common anaerobes and bifidobacteria are less common than the flora of healthy

breast fed term infants in whom bifidobacteria predominate **(**9**)**. Very low birth weight infants often have a paucity of normal enteric bacterial species **(**lactobacillus and bifidobacterium**)** and a delayed onset of colonization compared to term infants **(**21**)**. Since microbial invasion of the gut wall may be a contributing cause of NEC, altering microbial flora by enteral feeding of probiotics might be beneficial.

Bifidobacteria are gram positive anaerobic bacteria that colonise the intestinal tract of healthy breast feeding infants **(**22, 23**)**. These bacteria seem to be the predominant organism in breast fed infants, but they are less prevailing in formula fed and premature infants, who have the highest risk of NEC **(**24**)**. Along with lactobacilli, bifidobacteria have been shown to be beneficial in preventing or treating gastroenteritis, rotaviral diarrhea and other gastrointestinal diseases **(**25**)**. The benefit of bifidobacterial colonization may occur in past because of their ability to reduce local luminal PH and there by select against the growth of more pathogenic organisms such as E.coli **(**26**)**. In addition, bifidobacteria release little endotoxin and therefore less effectively stimulate the production of inflammatory mediators including interleukin 1 and 6 and TNF – alfa. These finding suggest that bifidobacterial colonization may play a protective role to reduce the development of neonatal NEC.

Masahiko Urao et al studied nine infants including five with biliary atresia, two with omphalocele, one each with hirshprungs disease and imperforate anus. A probiotic mixture was given for 2 weeks and fecal aerobic and anaerobic bacterial cultures , serum endotoxin levels were examined and was found that probiotics affect intestinal bacterial flora by increase in anaerobic bacteria and reduce the population of potentially pathogenic microorganism. A reduction in the luminal endotoxin may result in less endotoxin translocation , thereby preventing NEC. **(**27**)**

Aim of the study

To evaluate the efficacy and safety of probiotic supplementation **(**started on day one for total of 10 days**)** in preventing stage 2 or greater NEC in preterm neonates **(** gestation < 35 weeks **)** and birth weight of < 2 kgs.

Material and Methods

Type and place of study

Retrospective Cohort study at Manipal Hospital, Bangalore.

Period of study

1st December 2007 to 20th October 2009.

Inclusion Criteria

Preterm Neonates with gestational age of <35 weeks

Low birth weight < 2000 grams

Hemodynamically stable

Exclusion criteria

Congenital defects especially involving gastrointestinal tract

Gestational age: > 35 weeks.

Birth weight: > 2000 grams.

Materials used

Probiotic: Preprokid Sachet **(**Lactobacillus acidophilus 650 million, Lactobacillus rhamnosus 400 million, Bifidobacterium longum 100 million, Bifidobacterium infantis 100 million, Saccharomyces boulardii 50 million**)** Distilled water.

Methodology

Preterm neonates will be selected strictly based on inclusion and exclusion criteria. The baby will be administered preprokid sachets 250 million units per day twice daily mixed with distilled water under aseptic precautions for 10 days. All the babies will be monitored for increased or altered nasogastric aspirates, abdominal distension, vomiting and bloody stools. Standard practice guidelines as followed

for the care of these babies. In case of suspicion of NEC, the neonate will be managed appropriately and the drug will be stopped. Babies will also be monitored for any possible side effects of the drug.

Observation and analysis

A total of 193 preterm babies with a gestational age of < 35 weeks and birth weight of < 2000 grams were studied between 1st December 2007 to 20th October 2009. Probiotics was given to 62 preterm babies. All the babies were followed till the discharge from NICU and the results were tabulated and interpreted as follows.

Subjects and Methods

This is a retrospective cohort study of 193 preterm babies with a gestational age < 35 weeks and birth weight of < 2000 grams done at NICU, Manipal Hospital, Bangalore from December 2007 to October 2009. The study group included only inpatients babies admitted to NICU, Manipal Hospital, Bangalore.

All the preterm babies admitted to NICU, Manipal Hospital, Bangalore, were included in the study based on inclusion and exclusion criteria and was started on probiotics **(**preprokid sachet, one sachet diluted in 5 ml of sterile water and 1 ml was given twice daily for total of 10 days **)**. All the babies were monitored for increased and altered aspirates, abdominal distension, stool for occult blood. On admission to NICU septic work up which included complete blood count, C Reactive protein and Blood cultures were done for all the babies. When suspected NEC based on Bells criteria diagnosis was made , septic workup was repeated, sodium levels and X ray abdomen was done and an opinion from the Pediatric Surgeons was taken and managed accordingly

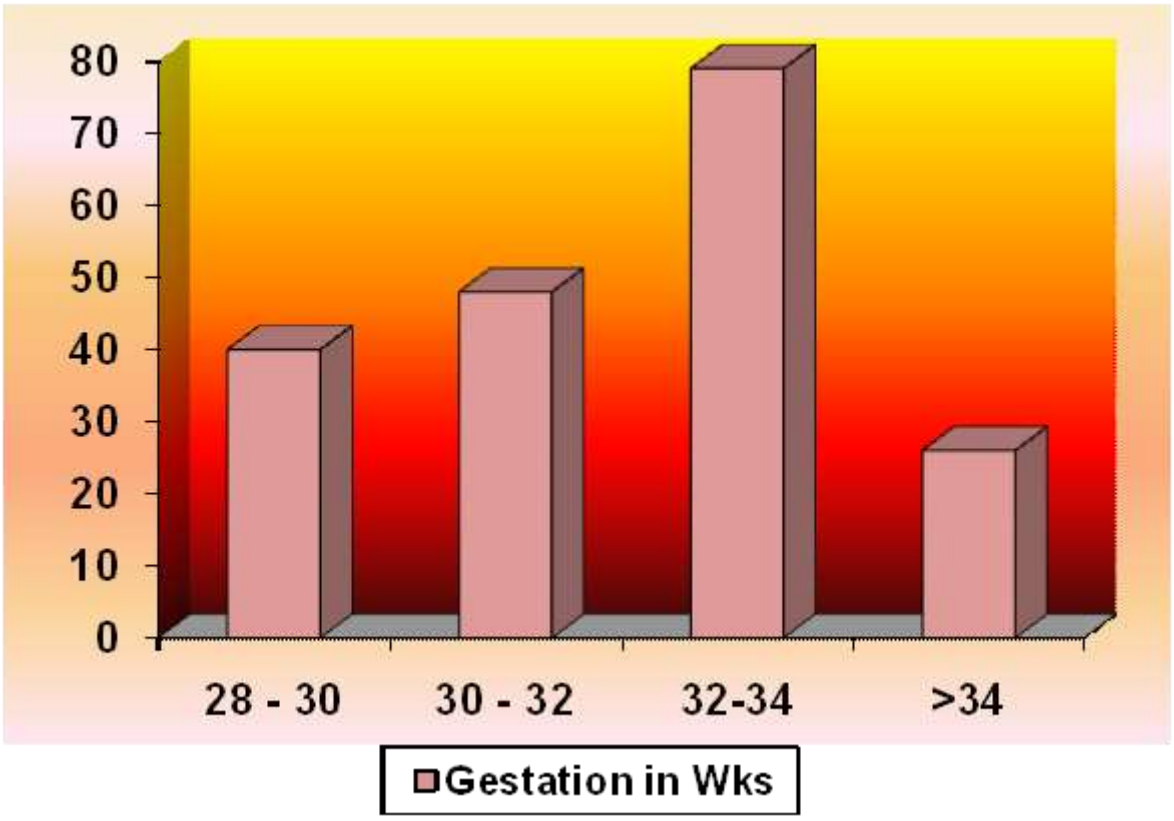
Study Design

This is a retrospective cohort study consisting of 193 preterm babies, included in the study based on inclusion and exclusion criteria to NICU, Manipal Hospital, Bangalore between December 2007 to October 2009.

Age Distribution of the study group

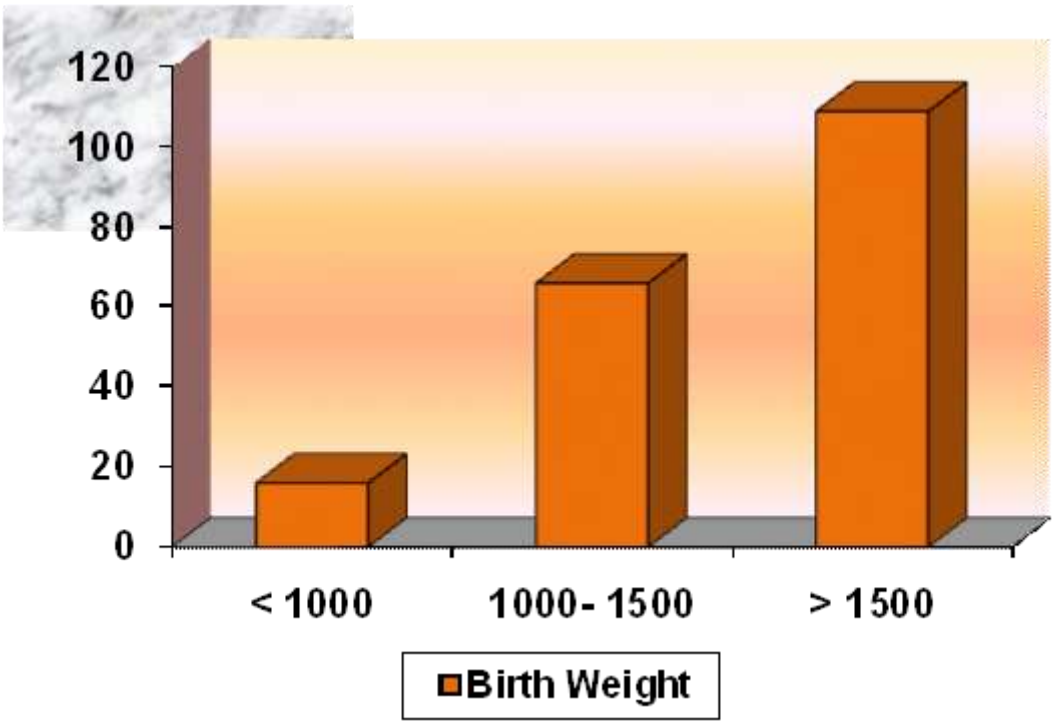
Gestational Age(weeks)	Number (n= 193)	Percentage (%)
28 - 30	40	20.7
31-32	48	24.9
33-34	79	40.9
>34	26	13.5
Total	193	100

Age distribution of the study group was analyzed. Out of 193 preterm babies studied, 40 babies were between 28-30 weeks of gestational age, 48 babies were between 31-32 weeks of gestational age, 79 babies were between 33-34 weeks and 26 babies were > 34 weeks of gestational age.



Birth Weight Distribution of the study group:

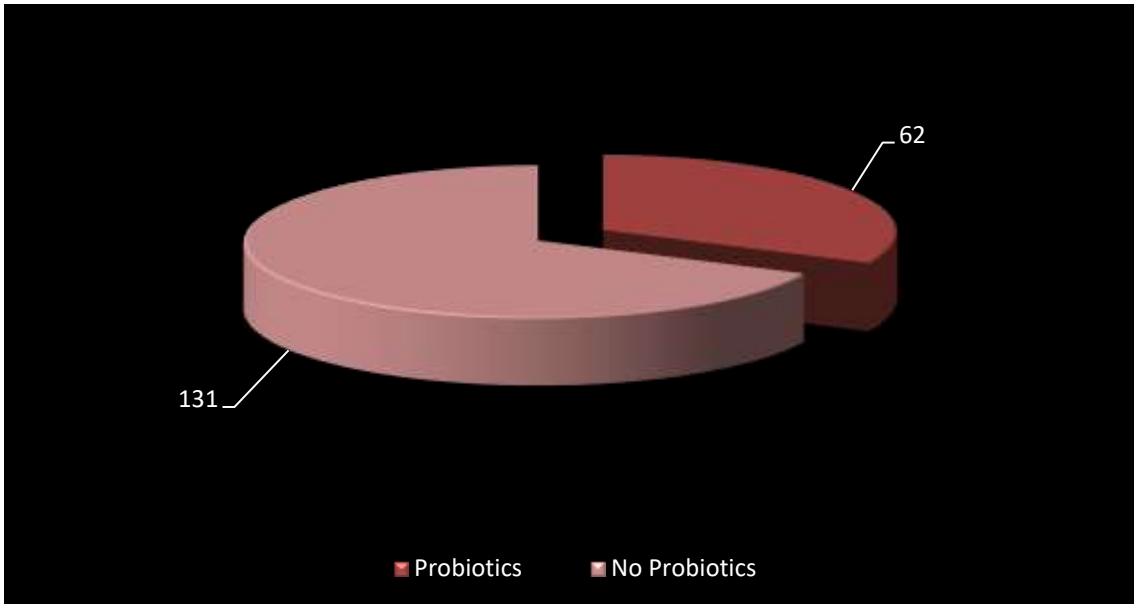
Birth Weight in grams	Number (n=193)	Percentage (%)
<1000	16	8.4
1001-1499	68	34.6
1500-2000	109	57.0
Total	193	100



Birth Weight distribution analyzed reveals that 57% of the preterm babies were with a birth weight between 1500-2000grams, 34.6% of the babies were between 1001-1499 grams and only 8.4% of the babies were < 1000 grams. The mean birth weight of our study group was 1514.27+/- 327.13 grams.

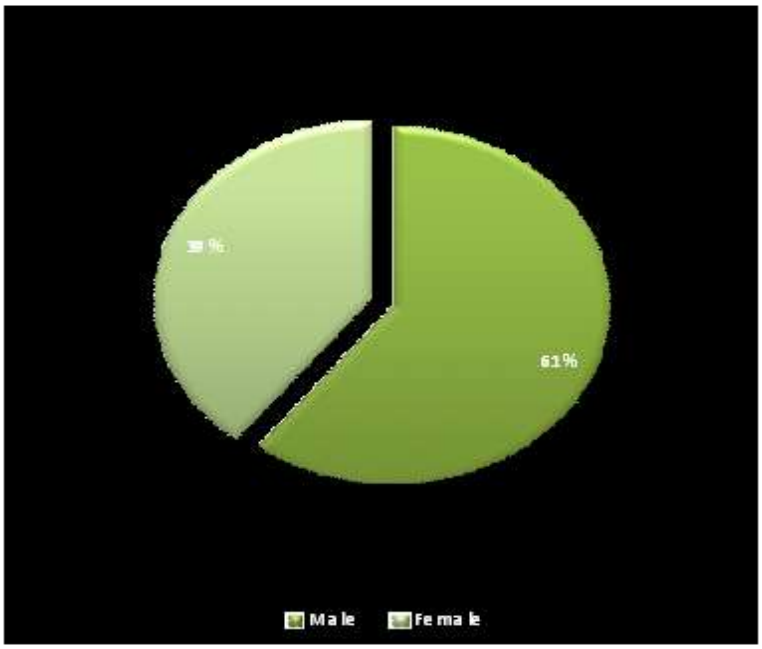
Study group receiving Probiotics

Probiotics	Number (n=193)	Percentage (%)
Yes	62	32.2
No	131	67.8
Total	193	100



Sex Distribution of the study group:

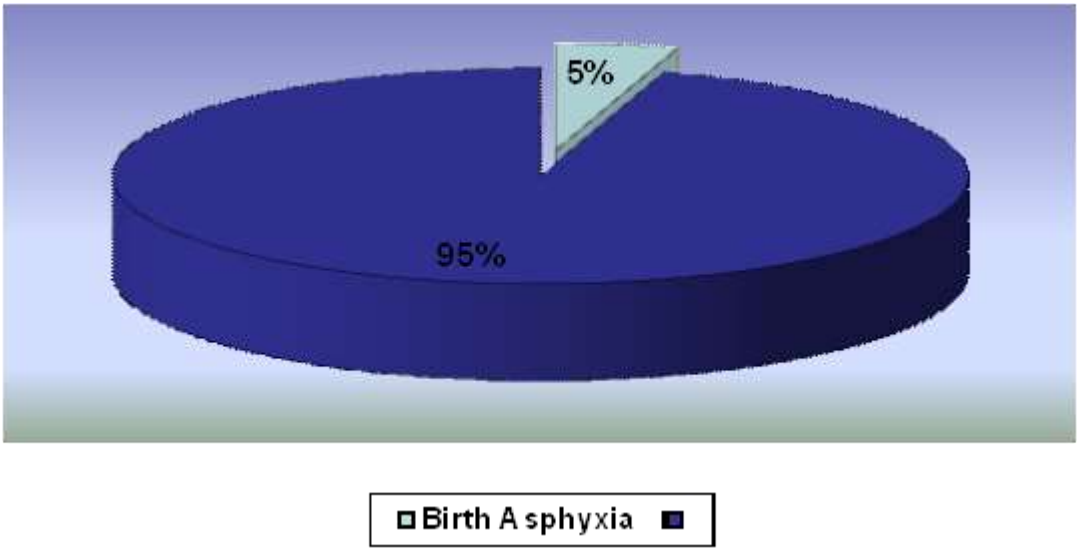
Sex	Number (n=193)	Percentage (%)
Male	117	60.6
Female	76	39.4
Total	193	100



Male to female distribution of the study group revealed that 60.6% were males and 39.4% were females.

Analysis of Birth Asphyxia:

Birth Asphyxia	Number (n=193)	Percentage (%)
Yes	9	4.7
No	184	95.3
Total	193	100

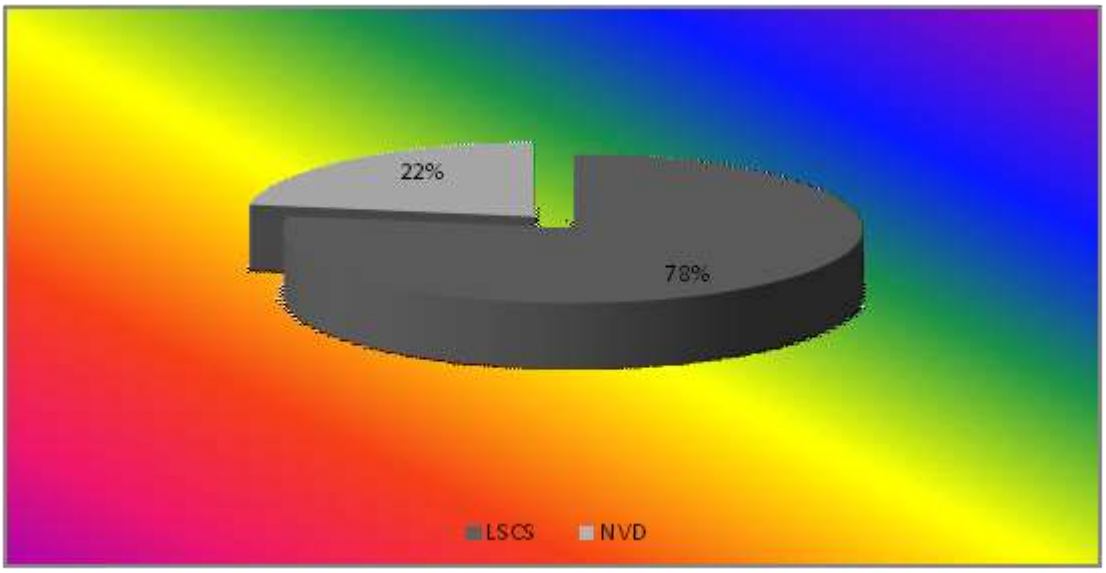


Birth Asphyxia is one of the risk factor in developing NEC. In our study group analysis of birth asphyxia 4.7% preterm babies had birth asphyxia. **(**Birth Asphyxia defined as Apgar score of </= 3 at first minute**)**. It was noticed that none of the babies with birth asphyxia had NEC and 4 babies among them had received probiotics.

Mode of Delivery:

Mode of Delivery	Number (n=193)	Percentage (%)
LSCS	150	77.6
NVD	43	22.4
Total	193	100

It was noticed that 150 preterm babies **(**77.6%**)** were delivered by LSCS and 43 babies **(**22.4%**)** were delivered by NVD



Analysis of initiation of feeds

Feeds Initiated on Day	Number (n=193)	Percentage (%)
1	44	23
2	86	45
3	42	22
4	9	4.7
5	5	2.6
6	3	1.6
7	1	0.5
8	1	0.5
Total	191	100

Initiating feeds early has shown to reduce the incidence of NEC in preterm babies. In our study group the mean time of initiating feeds was 2.25days with a standard deviation of +/- 1.17 days. 2 babies were not initiated on feeds at all, one baby developed illeal perforation, was operated for the same, expired due to NEC and multiorgan dysfunction failure and the other baby had meconeum plug and was operated for the same.

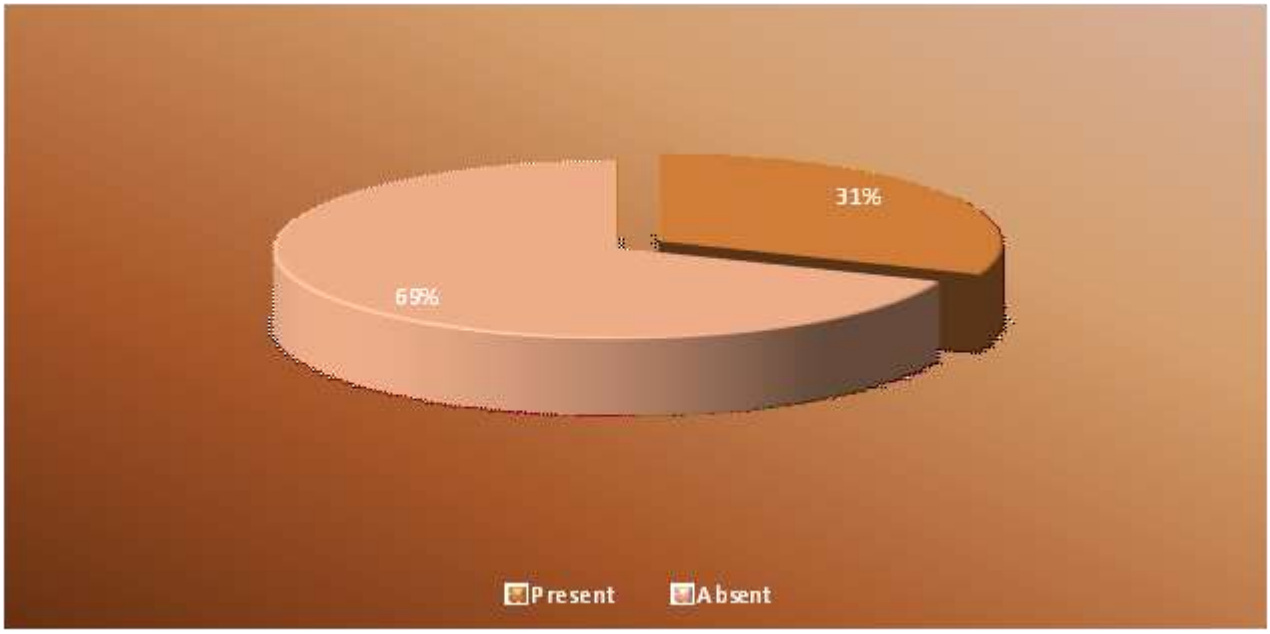
Analysis of Type of feed:

Type of feed	Number (n=193)	Percentage (5)	NEC
EBM	111	58	4
Formula	9	4.8	0
EBM+Formula	71	37.2	2
Total	191	100	6

One of the factor in reducing NEC is feeding the babies with breast milk. In the analysis of our study it has been noticed that 58% of the babies were fed with exclusively breast milk and 4 among them developed NEC. 37.2% were fed with both breast milk and formula and 2 among them developed NEC. Only 4.8% were fed with formula feed alone and none of the babies among them developed NEC.

Analysis of Naso-Gastric Aspirates and Abdominal distension

Nasogastric aspirate (increased & altered)	Number (n=193)	Percentage (%)	NEC
Yes	60	31.1	7
No	133	68.9	0
Total	193	100	7

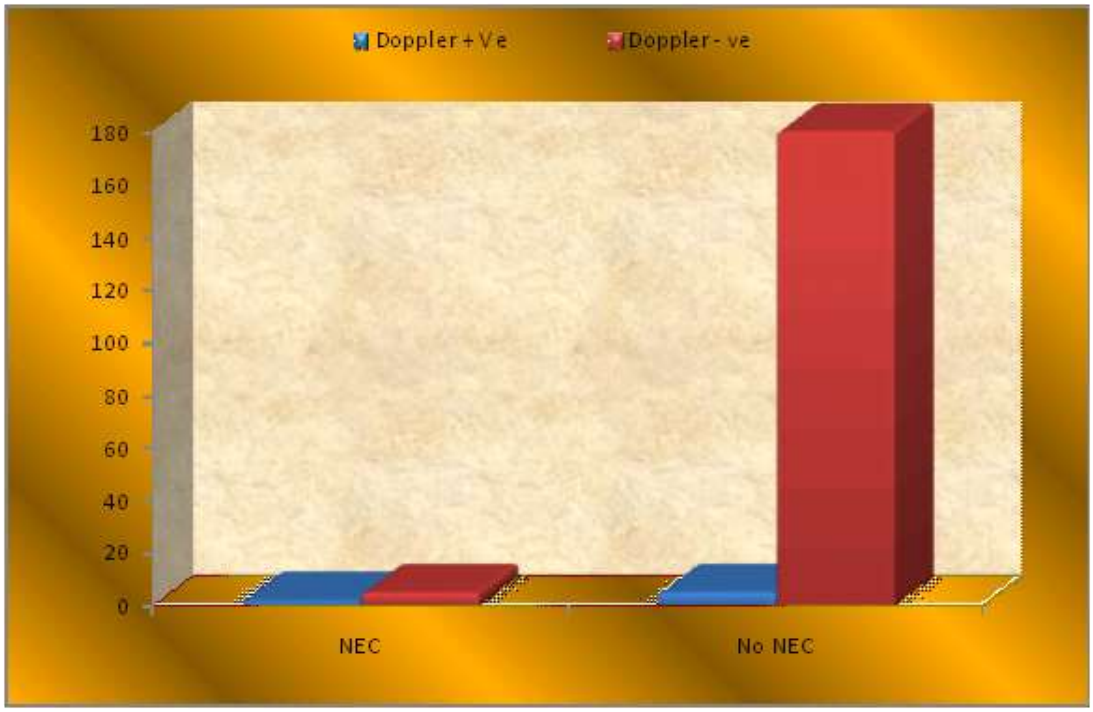


Abdominal distension	Number (n=193)	Percentage (%)	NEC
Yes	19	9.4	7
No	174	90.6	0
Total	193	100	7

Increased or altered Nasogastric aspirates and abdominal distension are the symptoms monitored for to suspect NEC. In our study we noticed that 31% had increased and altered aspirates and 9.4% developed abdominal distension. It was noticed that all the babies who developed NEC had both increased and altered aspirates and abdominal distension.

Antenatal Doppler changes and NEC analysis:

Antenatal Doppler changes	Number (n=193)	Percentage (%)	NEC	Percentage (%)
Yes	7	3.6	2	28.5
No	186	96.4	5	71.4
Total	193	100	7	100

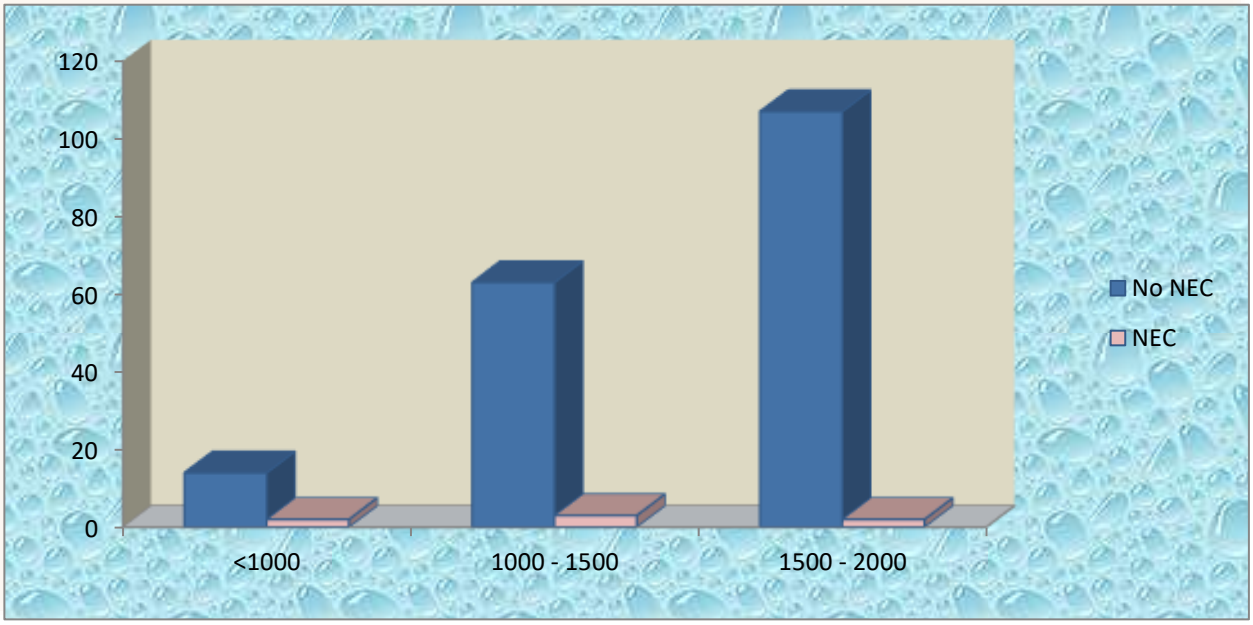


In our study we noticed that 3.6% preterm babies delivered had antenatal Doppler changes and among them 28.5% developed NEC compared to no NEC in 71.4% of the babies.

Analysis of Birth Weight and NEC:

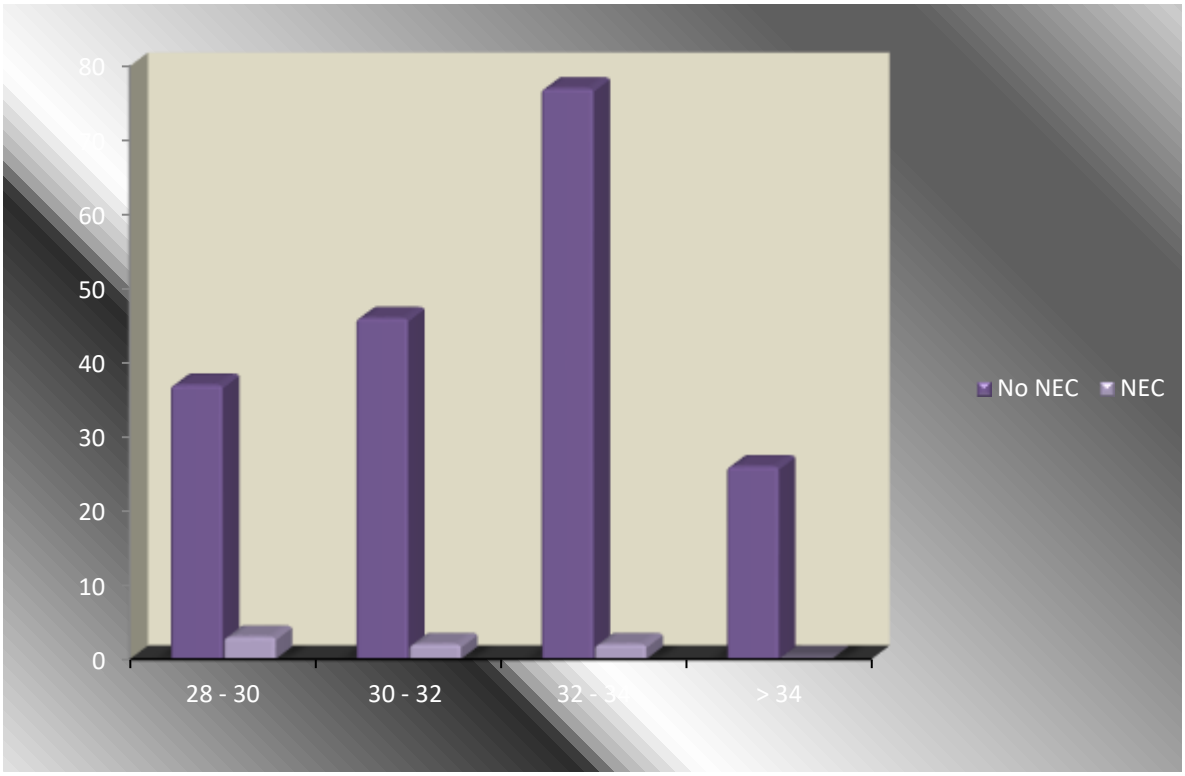
Birth Weight in grams	NEC positive	NEC Negative
<1000	2	14
1001-1499	3	65
1500-2000	2	107
Total	7	186

Analysis of birth weight and NEC revealed that 2 babies with a weight of < 1000grams, received probiotics developed NEC and both expired. Three babies weight between 1001-1499 grams developed NEC, among which 2 of them received probiotics and one expired. Two of the babies with birth weight >1500 grams developed NEC. The P Value being 0.095 is insignificant.



Analysis of gestational age and NEC:

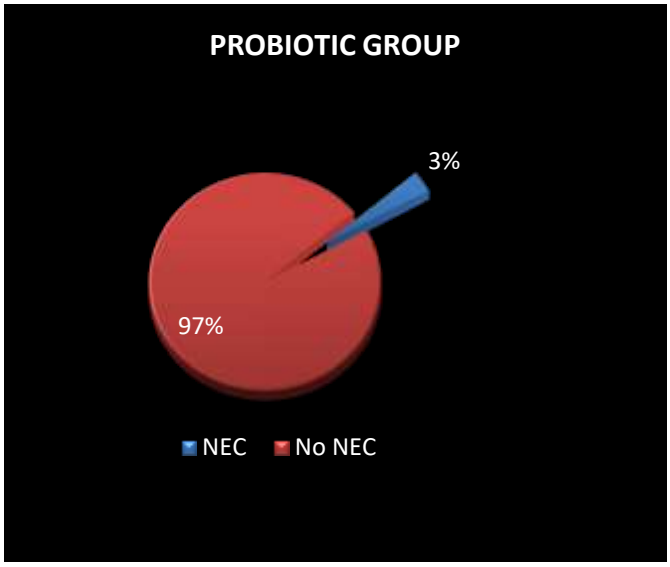
Gestational age in weeks	NEC
28-30	3
31-32	2
33-34	2
>34	0

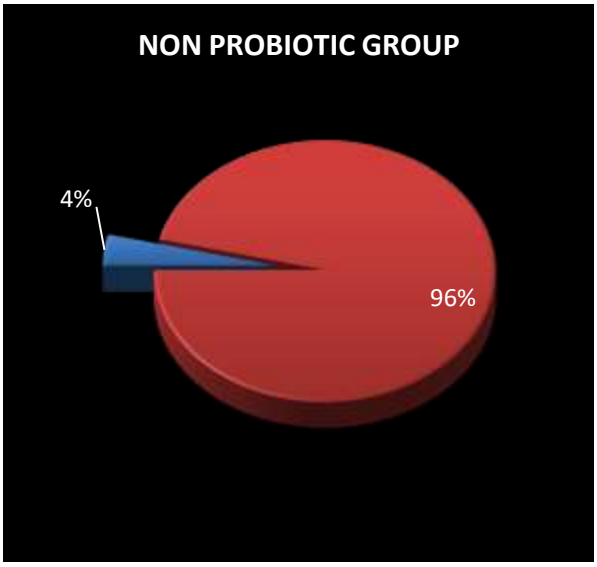


The less the gestational age, the more is the incidence of NEC in preterm babies. Analysis of gestational age and NEC in our study group revealed that 3 babies developed NEC between the gestational age group of 28-30 weeks, 2 babies between 31-32 weeks and 2 babies between 33-34 weeks and none with a gestational age of >34 weeks. The P Value being 0.39 is insignificant.

Analysis of Probiotics and NEC:

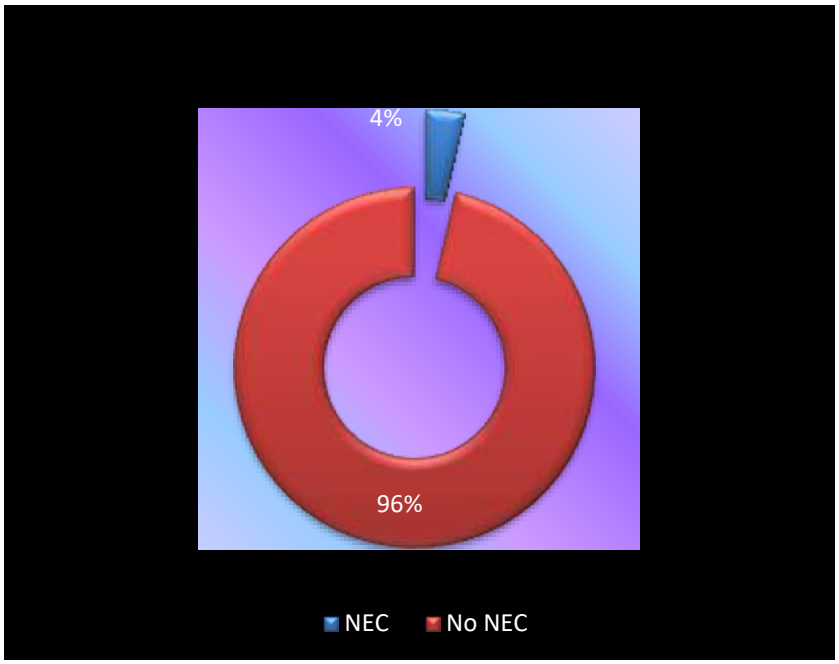
Probiotics	**Number of babies**	**NEC**
Yes	62	2
No	131	5
Total	193	7





The incidence of NEC in probiotic and control group was analysed. It was noticed that 3% of the probiotic group developed NEC and 4% of the control group developed NEC. The P Value is 0.8 which is insignificant.

NEC



Discussion

The consumption of probiotic agents such as lactic acid bacteria first occurred when humans began using fermented food stuffs. After observing the arrested putrefaction of milk products by lactic acid bacteria, Metchnikoff first proposed the use of probiotics to prolong human life in 1907. Fuller has defined a probiotic as a live microbial feed supplement, which beneficially affects the host animal by improving its microbial as balance. It is generally agreed that a probiotic must be able to resist the extremes of PH during gastrointestinal transit and be able to colonize, although not necessarily proliferate in the gut, to exert its potential beneficial effects.

Lactobacillus GG was reported in 1985 by Gorbach and Goldin as a natural strain **(**isolated from humans**)** having the properties of a candidate probiotic. LGG has been studied in a variety of clinical settings in different age groups and shown to be beneficial.

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