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PREBIOTICS AND PROBIOTICS IN PRETERM NEONATES

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LIST OF ABBREVIATIONS USED

1. NEC - Necrotising Enterocolitis
2. PT - Preterm
3. lBW - Low Birth Weight
4. VLBW - Very Low Birth Weight
5. NICU - Neonatal Intensive Care Unit
6. SGA - Small For Gestational Age
7. AGA - Appropriate For Gestational Age
8. LGA - Large For Gestational Age
9. GA - Gestational Age
10. GIT - Gastrointestinal Tract
11. IUGR - Intrauterine Growth Retardation

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1 INTRODUCTION

Premature or preterm infants are those born before 37 weeks' gestational age.1.The incidence of preterm births (< 37 weeks gestation) is increasing in many countries around the world and has become a global health concern amongst the developing countries India has a very high incidence of 22%.[2]

Low birth weight is associated with prematurity and defined as birth weight<2500g, Premature infants, especially very low birth weight (VLBW)(<1500gm) are at risk of neonatal morbidity and mortality, Immaturity of the organ systems of preterm infants makes them more susceptible to many complications including respiratory distress syndrome, feed intolerance, bronchopulmonary dysplasia, necrotizing enterocolitis (NEC), patent ductusarteriosus, sepsis, anemia, retinopathy of prematurity and intraventricular hemorrhage.[3]

In order to survive in extra uterine environment, preterm babies often need special care and extensive support in neonatal intensive care unit.Among all Necrotizing enterocolitis is one of the major causes of morbidity and mortality in neonatal intensive care units (NICU) which involve 7% to 14% of premature babies weighing less than 1500 gm.[4]

Prebiotic and probiotic have been identified in many studies to reduce the prevalence of necrotizing enterocolitis; feeding intolerance, sepsis, and improvement in the weight gain and reduce the mortality in preterm IUGR babies. Prebiotic and probiotics are easily available in market apart from; it is used for other condition like diarrhea and allergy.

Probiotics are supplement or foods that contain viable microorganism that alter the microflora of the host.Prebiotics are supplement or foods that contain non digestible ingredient that selectively stimulate the growth and /or activity of indigenous bacteria.[5]

The combination of probiotics and prebiotics is called symbiotic.The improvement of neonatal care has increased the survival rate of premature infants and consequently the incidence of feeding intolerance. The purpose of the book is to know the effects of probiotic and prebiotics on feeding intolerance, necrotizing enterocolitis, sepsis and weight gain in premature and IUGR babies in tertiary care hospital.

2 LITERATURE REVIEW

Preterm:Preterm birth is defined as birth prior to 37 completed weeks of gestation.[1]

Incidence- Approximately 12.7 % all births in United States are preterm .The distribution of this group is gradually shifting to relatively older gestational age because of a 25 % increase in late preterm infants (34-36weeks) since 1990 to current rate of 9.1%.[6]

Nearly 30% of neonates—7.5 million—are born with a LBW (<2500 g) in India.7This accounts for 42% of the global burden, the largest for any country. About 60% of the LBW infants are born at term after fetal growth restriction, whereas the remaining 40% are born preterm.8 The prevalence of SGA is 46.9%, higher than all but two countries in the world (Pakistan and Mauritania have a marginally higher prevalence at 47.0%.7Each year, ~ 3.5 million preterm (<38 weeks of gestation) neonates are born in India.7 Community-based studies indicate that the LBW infants are at 11– 13 times increased risk of dying than NBW infants.43 Indeed, 480% of total neonatal deaths occur among LBW/preterm neonates.[9,10]

Definition of weight of baby based on gestational age.6

Small for gestational age-(SGA)

Defined as weight below the 10th percentile for the period of gestation.

Large for gestational age-(LGA)

Defined as a weight, length, or head circumference that lies above the 90th percentile for that gestational age.

Appropriate for gestational age-(AGA)

Defined as weight, length, and head circumference that lies in between 10th percentile and 90th percentile for that age.

ETIOLOGY OF PRETERM -

- **Low social economic status-** measured by family income, education status, geographical area, social class and occupation.
- **Non-Hispanic black-Women** are more than three times as likely to deliver an extremely preterm infants (<28 weeks of gestation) (1.9%) compared with non-Hispanic white and Hispanic women (0.6%).
- **Women younger than 16 or older than 35 more** likely to deliver preterm or LBW infants; the association with age is more significant in whites than in African Americans.
- **Maternal activity** requiring long periods of standing or substantial amounts of physical stress maybe associated with IUGR and prematurity.
- **Acute or chronic maternal illness** is associated with early delivery, whether spontaneous or not infrequently, induced.
- **Multiple gestation births** frequently deliver preterm (60% of twins and 94 % of triplets in United States in 2005).in such births higher rate of neonatal mortality is primarily due to prematurity.
- **Prior poor birth outcome** is the single strongest predictor of poor birth outcome. A preterm first birth is the best predictor of second preterm birth.
- **Obstetric factors** such as uterine-malformations, uterine-trauma, placenta Previa, abruptio placentae, hypertensive disorder, preterm cervical shortening, previous cervical surgery , PROM and chorioamnionitis also contribute to maternal factors.
- **Fetal conditions** such as non-reassuring testing of fetal well-being, IUGR, or sever hydrops may require preterm delivery.
- **Inadvertent early delivery** because of in correct estimation of GA is increasingly uncommon.[6]

The preterm gastrointestinal system

Aside from its digestive and absorptive functions, the gastrointestinal (GI) tract is an essential immune organ and the largest defense barrier protecting the host from pathogens, toxins and subsequent inflammation while allowing commensal bacteria to grow.

The GI tract begins to develop at four weeks' GA, facilitated by amniotic fluid, and continues to mature throughout childhood under the influence of dietary and environmental factors.11The maturity of this

system is directly proportional to GA. The preterm infant's gut is immature in multiple functions including motility, digestion, barrier defense function, intestinal permeability, immune defense and anti-inflammatory control.12The immaturity of these functions can lead to significant pathological symptoms and complications such as feeding intolerance due to dysmotility and bacterial translocation – a phenomenon in which bacteria cross the 'leaky gut' of the premature infant and spread into lymph and blood, causing sepsis and multiorgan failure.[13]

In addition, the production of digestive enzymes, mucus and immunoglobulins is inadequate, which can allow pathogenic invasion and intestinal injury. Furthermore, preterm infants in the neonatal intensive care unit experience delayed initiation of enteral feeding and are exposed to common medications, such as antibiotics and H2 blockers, all of which cause intestinal atrophy and alter the defense barrier and immunity, allowing epithelial adherence and bacterial translocation.[14]

The micro biome is a complex ecosystem consisting of more than 1000 species of live bacteria that play major roles in nutrition and in the development of the immune system.15The development of the neonatal micro biome begins with the exposure of the fetus to microbes in the amniotic fluid.16 and continues to diversify depending on factors such as GA, mode of delivery, hospitalization, antibiotic use and type of feeding.[17]

Unlike the micro biome of the term infant, the preterm infant micro biome is less diverse and is predominated by Staphylococcus species, with Bifido bacterium species being less well represented.18This is due to the fact that preterm infants are primarily treated with a course of broad spectrum antibiotics.19Another cause of disequilibrium in the intestinal micro biota is bacterial colonization from the intensive care environment.20 These changes in the composition of the micro biome of the preterm infant can further alter the development of epithelial barrier mechanisms and gut immune function.

Accumulating evidence has shown that imbalances in intestinal micro biota may enhance certain acute diseases, such as neonatal sepsis and

NEC, and may be involved in the initiation of chronic diseases such as type I diabetes, inflammatory bowel disease and obesity.[21-23]

Table No 1: The common problems associated with prematurity mentioned below

General	Hypothermia, trans epidermal fluid loss
CNS	Apnea, intraventricular bleed, birth asphyxia
Lung	Respiratory distress syndrome, chronic lung disease,
CVS	PDA,
GIT	Feed intolerance, NEC, cholestasis, intraabdominalbleed, and hyperbilirubinemia.
Hematology	Anemia of prematurity
Immune system	Infections(bacterial,viral,fungal)
Eye	Retinopathy of prematurity(ROP)
Metabolic	Hypoglycemia, hyperglycemia, hypocalcemia, hyperkalemia, acidosis

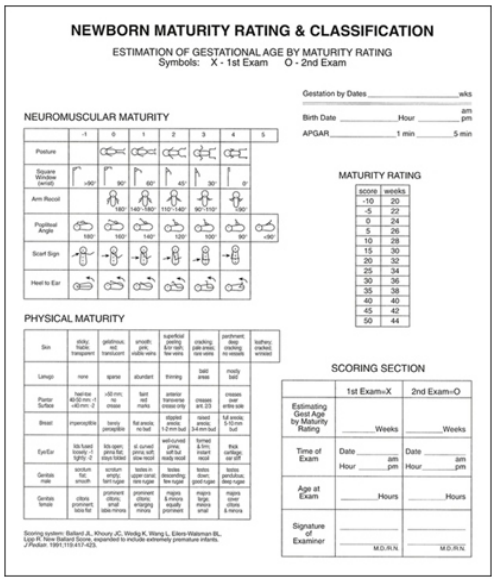

Table No 2 : ASSESSMENT OF PRETERM BABIES- BASED ON BALLARD SCORING SYSTEM

NECROTIZING ENTEROCOLITIS-

Definition –

NEC is an acute inflammatory necrosis of gastrointestinal tract.

Necrotizing enterocolitis is one of the major causes of morbidity and mortality in neonatal intensive care units (NICU) which involve 7% to 14% of premature babies weighing less than 1500g.4The National Healthcare Safety Network (NHSN) updated the clinical and surgical criteria for the diagnosis of NEC.

These updated criteria stated that for an infant to be diagnosed with NEC based on clinical and radiographic criteria, they must present with bilious aspirate, vomiting, abdominal distension, or occult blood in stool, plus one or more of the following radiographic signs: pneumatosisintestinalis, which is gas in the bowel wall, portal venous gas, or pneumoperitoneum.[24]

Furthermore, the surgical criteria for NEC are that the infant must have more than two centimeters of necrotic bowel or surgical evidence of pneumatosisintestinalis, with or without intestinal perforation.[24]

It has been hypothesized that NEC results from the interaction between prematurity and hypoxic ischemic events in the perinatal period, which include low Apgar score, enteral feeding, episodes of apnea and administration of Indomethacin.25 Although the exact etiology of NEC is not well understood, pathogenesis is believed to be due to a multifactorial process that is related to one or more of the following:

- Hypoxic ischemic events
- Immaturity and dysfunctionality of the GI tract (e.g. impaired peristalsis and disruption of tight junctions)
- Altered micro biota and
- Enteral feeding.[26]

The net result of the interaction among these factors is the invasion of the intestinal wall by bacteria, followed by bacterial translocation and release of inflammatory mediators. Other factors that may contribute to the development of NEC include-

- Insufficient production of epidermal growth factor, an enzyme responsible for cell proliferation and differentiation
- Additional stimulation of platelet-activating factor, a phospholipid inflammatory mediator
- ncreased production of nitric oxide, a vasodilator and free radical molecule.[27,28]

During hypoxic ischemic events, it has been postulated that blood is shunted from the bowel to vital organs, such as the brain, and the reperfusion of blood to the intestine provokes a pro-inflammatory cytokine cascade in the gut.[29] The release of pro-inflammatory cytokines further disrupts tight junctions, causing an increase in intestinal permeability and,therefore, bacterial translocation.[29],[30] Moreover impairedperistalsis of the preterm intestine may allow more time for the carbohydrate from enteral feeding to serve as bacterial substrate, thereby leading to bacterial invasion of the intestinal wall and inflammation.

Another important factor is the compromise of serum immunoglobulin A production, which eventually eases bacterial translocation.[29],[30]The use of antibiotics, in addition to the previously mentioned factors, facilitates the proliferation of pathogenic bacteria, which may then induce a hyper immune inflammatory response in the preterm infant intestine causing intestinal necrosis.[29] The use of breast milk and probiotics are potential preventive strategies to reduce the incidence of this devastating complication.[28]

Table No 3 : DIAGNOSIS AND CLASSIFICATION OF NECROTIZING ENTEROCOLITIS BASED ON BELL'S STAGING.

Stage	Classification	Intestinal Signs	Radiologic Signs	Radiologic Signs
IA	Suspected NEC	Decreased gastric emptying, Abdominal distention, Emesis	Normal or Intestinal obstruction	Temperature instability, apnea, Bradycardia, lethargy

IB	Suspected NEC	Bright red blood from rectum	Same as IA	Same as IA
IIA	Proven NEC-mild	Same as IA plus Absent bowel sou nd, with or without abdominal tenderness	Intestinal dilatation ,ileus, and, Pneumatosi sintestinalis	Same as IA
IB	Suspected NEC	Bright red blood from rectum	Same as IA	Same as IA
IIA	Proven NEC-mild	Same as IA plus Absent bowel sound,with or without abdominal tenderness	Intestinal dilatation , ileus, and, Pneumato sisintestinalis	Same as IA
IIB	Proven NEC-moderate	Same as IIA withDefinite abdominal Tenderness and with or withoutright lower lobe quadrant mass	Same as IIA plusportal venous gas , with or without ascites	Same as IA plus mild metabolic acidosis and thromboc ytopenia
IIIA	Advanced NEC bowelintact Requires surgery.	Same as IIB withgeneralized peritonitis, marked tenderness,and distention of abdomen	Same as IIB with definite ascites	Same as IIB with hypotension ,bradycardia, apnea, respiratory and metabolic acidosis and neutropenia

IIB	Advanced NEC bowelperforation Requires Surgery.	Same as IIIA	Same as IIB with Pneumope ritoneum	Same as IIIA

TREATMENT OF NECROTIZING ENTEROCOLITIS

Treatment of NEC ranges from bowel rest for suspected NEC, the use of antibiotics alongwith bowel rest for proven but mild NEC, to surgery for advanced NEC. When NEC progressesinto the "advanced" stages of NEC and requires surgery, it is termed "surgical NEC" and theLong-term prognosis for the infant decreases. Long-term ramifications of surgical NEC includeShort bowel syndrome and neurodevelopment impairment (NDI).[31]Due to its costly and deadly nature, an effective preventative strategyneeds to be further researched and implemented.[32.]

There are multiple proposed strategies for the prevention of NEC. These strategiesinclude antenatal corticosteroids, which have been shown to mature the gut in a manner similarto the mechanism enhancing lung maturation; trophic feedings in which small volumes of enteralfeedings are introduced in order to facilitate peristaltic action; oral antibacterial, in an effort toreduce the number of pathogenic bacteria; and prebiotics, used to selectively increase thepopulation of commensal GI bacteria. And lastly, the use of probiotics, thought to be the mostpromising of therapies, and the focus of this literature review.[33]

NEONATAL SEPTICEMIA

Neonatal sepsis is defined as a clinical syndrome of bacteremia with systemic signs and symptoms 0f infection in first four weeks of life. Neonatal septicemia is the most importantcause of morbidity and mortality especially among LBW and preterm babies in developing countries.When clinical and laboratory findings are consistent with bacterial infection but blood culture is sterile,infant is labelled to have "probable sepsis" .

According to NNPD (neonatal-perinatal data) the incidence of neonatal sepsis is around 30 per 1000 live births. Neonatal sepsis is

divided in to two subtypes based on whether the onset is during first 72 hrs. or later.

Early onset of sepsis is caused by the organism mainly present in the genital tract or in the labor room and maternity operation theater.in west most prevalent organism is Group B streptococci and E coli,while in our setup most cases are due to gram negative organism especially E.coli, Klebsiella,and Enterobacter species.

When two or more fallowing high risk factors present, the baby is considered to be treated with appropriate antibiotics.

- Pr esence of foul smelling liquor
- Febrile illness in mother during or within two weeks of delivery
- Prolonged rupture of membrane (>18hrs).
- Single unclean or more than three veginal examinations during labor.
- Prolonged labor (>24hrs both stages) and difficult delivery with instrumentation.
- Birth asphyxia and difficult resuscitation.
- Pathological evidence of funisitis or presence of polymorphs (>5/HPF) in gastric aspirate.

Late onset of sepsis defined as the onset of sepsis is delayed for 72 hrs after birth. Mainly organism acquired from the nursery or postnatal ward.

Most common organism responsible are Gram negative bacilli viz. Klebsiella pneumonia, enterobacteriaE.coli, pseudomonas aeruginosa, Salmonella typhimurium, Proteus sp,, Citrobacter and serratia while the rest are contributed by Gram positive organism including coagulase positive staphylococcus aureus and coagulase negative staphylococci.

The manifestations of neonatal septicemia are often vague and therefore high index of suspicion for early diagnosis(table no).the most common and characteristic manifestation is an alteration established feeding behavior in late onset sepsis and respiratory distress in early onset.

Table No 4 : Clinical manifestations

Lethargy Refusal of feed Poor cry Abdominal distention Diarrhea Vomiting Hypothermia Poor perfusion Sclerema Shock Bleeding Renal failure	Cyanosis* Tachypnea* Chest retractions* Grunting* Apnea/gasping* Seizures+ Blank look+ High pitched cry+ Irritability+ Bulging fontanelle+ Neck retractions+

*Particularly suggestive of pneumonia.
+particularly suggestive of meningitis.

Table No 5 : Diagnosis and screening of neonatal sepsis

Parameter	Abnormal values
ParameterTotal count Absolute neutrophil count Immature or band cell to total neutrophil ratio Micro-ESR C-reactive protein Blood culture	<5000mm3 Low count as per Manroe chart for term and Mouzhino chart for VLBW babies >0.2 >10mm 1sthr >1mg/dl Positive

- Sepsis screen is consider to be positive when 2 or more parameters are positive
- When initial screen is negative, it should be repeated after 12-24hrs when clinical suspicion of infections strong. when repeat sepsis screen is also negative , the diagnosis of sepsis can be excluded with reasonable certainty.

- In early onset sepsis , polymorphs in the gastric aspirate as a marker of chorioamnionitis, can be used as an additional parameter of sepsis screen
- Culture should be taken before starting the antibiotic therapy. And blood culture is considered to be gold standard for diagnosis of sepsis but it is positive only in 60% of patients.
- Lumbar puncture should always be done in a suspected case of late onset of sepsis except when the infant is too sick to undergo the procedure.

Management of neonatal sepsis

The rational use of antimicrobial agents in neonatal sepsis is governed by the knowledge of the prevalent bacterial flora of particular newborn NICU and there sensitivity battle against available antibiotics. The initial regimen must cover the most common pathogens. It should be borne in mind that there can be no single universal recommendation for the antimicrobial regimen.

Each treating unit should adopt a suitable protocol on the basis of considerations highlighted above. Based on changes in the spectrum ofetiologic agents. In the rural setting where antimicrobial resistance is less likely to be a problem, rational choice of antibiotic would include a combination of kanamycin or gentamicin (aminoglycoside) with Benzyl penicillin or ampicillin. Gentamicin 4mg/kg single-dose per day intramuscularly is effective for ambulatory management of neonatal sepsis in the community.

The most common organisms causing Sepsis in most in NICU are E. coli, Klebsiella, Enterobacter and Staphylococcus aureus. The initial antibiotic regimen must cover these pathogens. The logical initial choice would be a combination of an Aminoglycoside and Ampicillin or Cefazolinor Cloxacillin. The new were antibiotic preparation, like Tobramycin, Netilmicin, Vancomycin, cefotaxime, Ceftriaxone, Ceftazidime,Cefeperazone, Cefepime, and Imipenem should be kept in mind for treatment of meningitis and life-threatening infections. In centers with high incidence of resistance to third generation Cephalosporins and emergence of extended spectrum beta lactamase positive organisms, Piperacillin -Tazobactum or Methicillin-Vancomycin are drug of choice.

Ciprofloxac in should be used in a last resort in critically sick babies when bacterial isolates are resistance to all other antibiotics.

In desperate situations, newer antibiotics like Azitronam, andMeropenemmay be used. Is it true name has excellent activity against gram negative organisms while Meropenem is effective against most bacterial and pathogens except methicillin resistant staph aureus andEnterococcus .Imipenem is generally avoided in newborn baby is due to risk of seizures.

Table No 6 : Duration of antibiotic therapy in neonatal sepsis

Diagnosis	duration
Culture and sepsis screen negative but clinical picture issuggestive of sepsis	5-7 days
Sepsis screen is positive blood/CSF culture negative	7-10 days
Blood culture positive but no meningitis	10-14 days
Meningitis (irrespective of culture report)	21 days
Arthritis, osteomyelitis and endocarditis	4-6 weeks
Ventriculitis	6 weeks
*efforts should be made to administer antibiotics intravenously as long as feasible	

IMMUNOTHERAPY

Exchange blood transfusion in infected babies can theoretically help to achieve improved peripheral and pulmonary confusion, correction of coagulation abnormalities and removal of toxins; and provide specific antibodies, compliment and phagocytic cells,. The procedure is recommended in critical sick babies with Sclerema, DIC and Hyperbilirubinemia. Controlled studies are, however needed to further evaluated the therapeutic utility of exchange blood transfusion.

Granulocyte transfusion (1*109granulocytes/kg) is recommended as an adjunct to immunologic therapy for septic newborn infants with

neutropenia and has been used successfully in a limited number of infected babies to decrease the mortality.

Immunoglobulin preparations containing type-specific monoclonal antibody is to group the Group B Streptococci have been shown to be beneficial. There is evidence to suggest that administration of single dose of non-specific IVIG (750 mg/kg) in critically sick preterm infants (1gm/kg for term) with sepsis is associated with improved survival. In future specific immunoglobulins harnessed in donors or produced by monoclonal antibody technic are likely to be used.

Table No 7 : Complication

Meningitis Pneumonia Pyelonephritis DIC	Osteomyelitis and septic arthritis Shock Sclerema NEC

Prognosis-

Outcome depends upon weight and maturity of the infant, type of etiologic agent and it's antibiotic sensitivity pattern; and adequacy of specific and supportive therapy. Associated congenital malformations light meningomyeloceletracheoesophageal fistula and surgical procedure, adversely affecting prognosis. The early onset septicemia due to Group B Streptococcus and nosocomial infections due to Klebsiella and Pseudomonas Aeruginosa are associated with adverse outcome . Early and aggressive therapy is mandatory for improved salvage because extension of infection into various body organs and development of complications such as EndotoxicShoock, Sclerema, NEC, and DIC is associated with extremely high mortality.

The reported mortality rates in neonatal sepsis in various studies from India range between 15 and 50%. Early institution of specific antimicrobial therapy with the help of sepsis screen, excellent supportive care, close monitoring of vital signs, and judicious use of fresh blood, FFP and immunotherapy is likely to improve their outcome of units with septicemia. [34].

Feed intolerance-

Definition-

The inability to digest enteral feedings presented as gastric residual volume more than 50%, abdominal distention or emesis of both, and the disruption of the patient's feeding plan.[35]

Feeding intolerance is one of the most important factors of growth failure in preterm infants.Establishing and tolerating adequate enteral nutrition is difficult due to the immaturity of the preterm infants' gastrointestinal system; however, it is important for their normal growth, infection resistance, and long-term cognitive and neurologic development.

Clinical evidence of feeding tolerance in the preterm infant was most often described in the literature as the number of days required to reach full-feeding volumes (reported ranges from 100 to 160 millimeters per kilogram per day), the number of episodes of feeding intolerance, the number of days feeds are withheld due to feeding intolerance symptoms, time to regain birth weight, lower leg growth, and increase in weight gain, occipital-frontal head circumference, and length.[36,37]

Feed intolerance usually associated with fallowing symptoms like gastric residuals, emesis, abdominal distention, visible bowel loops, and blood in stool.

Apnea, bradycardia, and temperature instability are also included as symptoms of feeding intolerancebut solely for the purposes of the nursing assessment in order to provide guidance on identification of potential progression to more serious complications such as pneumatosisintestinalis and necrotizing enterocolitis.35

There are number of studies which show that prebiotic and probiotic therapy in prevention and treatment of feed intolerance.

MECHANISMOF ACTION OF PREBIOTIC AND PROBIOTIC IN PREVENTION OF FEED INTOLERANCE

Probiotic bacteria improve health by affecting the immune system in different ways. It increases cytokine production such as Interleukin-6 (IL-6), Interferon- gamma (IFN-γ), Tissue Necrosis Factor – alpha (TNF-α), Interleukin-1beta (IL-1β) and Interleukin-10 (IL-10).[38]some strains increase phagocytic activity of peripheral blood leukocytes (monocytes, polymorph nuclear cells).

Other strains strengthen the mucosal barrier function by promoting the production of mucosal antibodies and reducing the trans mucosal transfer of antigens. This reduces the intestinal permeability which in turn promotes growth.[39,40,41,42]

Probiotics bacteria also enhance production of low molecular weight antibacterial substances produced by epithelial cells and production of short chain fatty acids, the main energy source for colonocytes. This maintains the integrity of colon mucosa.[39,43,44,,45,46]

Probiotic

Definition –
Probiotics are supplements or foods that contain viable microorganisms that alter the microflora of the host.

The Greek meaning of the word probiotic is for life. Which are viable live microorganisms when administered in adequate amounts confer a health benefit on the host.Severallactococci, lactobacilli and bifid bacteria are held to be health benefiting bacteria but little is known about the probiotic mechanism of gut microbiota.[47]

Lactic acid bacteria or LAB constitute an integral part of the healthy gastrointestinal microceology and are involved in the host metabolism.[48]Fermentation has been specified as a mechanism of probiotics.[47]Probiotics along with other gut microbiota ferment various substrates like lactose, biogenic biogenic amines and allergenic compounds into short chain fatty acids and other organic acids and gases.[47,49,50].

LAB synthesizes enzymes, vitamins, antioxidants and bacteriocins.[48,51] With these properties, intestinal LAB constitutes an important mechanism for the metabolism and detoxification of foreign substances entering the body.[52]

Probiotics - properties

Probiotics have been suggested to have the following properties and functions:-

Adherence to host epithelial tissue, acid resistance and bile tolerance, elimination of pathogens or reduction in pathogenic adherence ,production of acids, hydrogen peroxide and bacteriocins antagonistic to pathogen growth, safety, nonpathogenic and non-carcinogenic, and Improvement of intestinal microflora.[53]

Influencing factors for the functionality of probiotics-

Several factors are there, which technically support and influencing the function of probiotics. Among them most important are strain characteristics, stability, fermentation technology, target prebiotics, viability and non-viability, microencapsulation etc.

Mechanisms of Action-

In preterm infants, probiotic supplementation can allow acquisition of normal commensal flora in a host where this process has been delayed or support the transition to an intestinal microbiome with beneficial microbes, particularly in hosts where this process has been disrupted.Several mechanisms of probiotic action may explain how their therapeutic use can help prevent NEC.

These mechanisms include enhancement of epithelial barrier function, competitive exclusion of pathogens, and direct anti-inflammatory effects on epithelial signaling pathways.[54–56]

At the cellular level, probiotics have a number of important effects (Figure 2)

1) Attenuation of NF-κB activation, a major pro-inflammatory pathway.[57]
2) Upregulation of cytoprotective genes.[58,59]
3) Prevention of apoptosis and cell death.[59,60]
4) Generation of reactive oxygen species important in cell signaling.[61,62]
5) Induction of the expression of tight junction proteins necessary for barrier function.[63,64]

Whether live microorganisms, instead of killed or inactivated bacteria or bacterial products, are required for these beneficial effects remains an important area of study and recent data suggest that bacterial products, in the absence of viable organisms, may have similar effects on signaling pathways.65 and barrier function.[63]

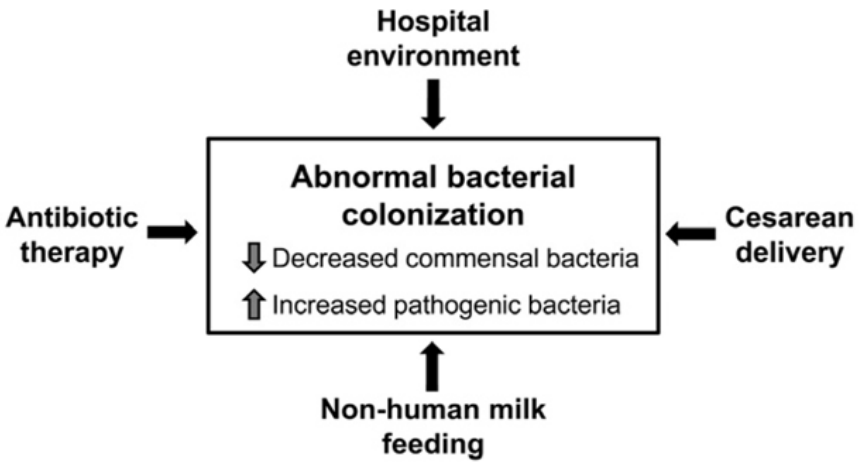

Figure 1. Factors influencing abnormal intestinal bacterial colonization in preterm infants

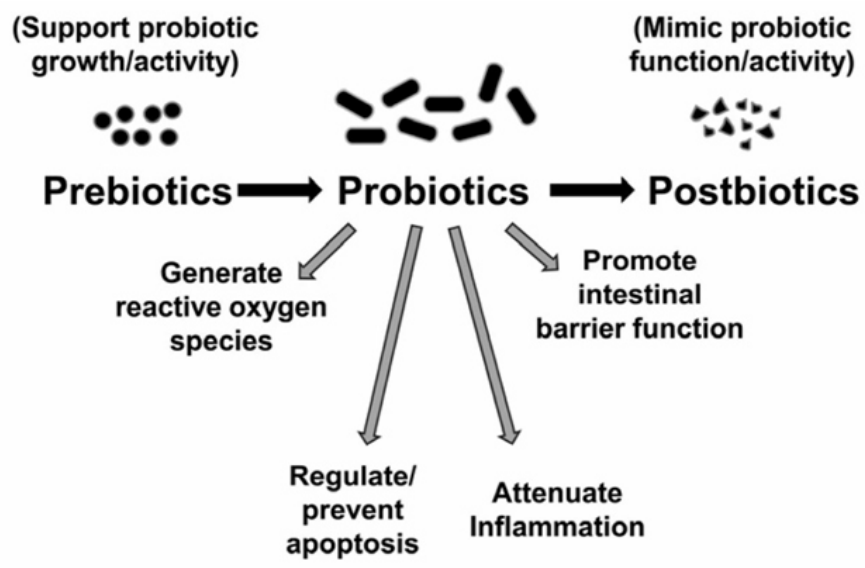

Figure 2. Mechanisms of action of probiotics at the cellular level in intestinal epithelia.

Prebiotics-

Definition-
Prebiotics are supplements or foods that contain a non-digestible ingredient that selectively stimulates the growth and/or activity of indigenous bacteria.

The concept of prebiotics came to light during mid-nineties of the twentieth century.[66]Prebiotics pass through the digestive system without being broken down by the digestive enzymes i.e. reach the large intestine in an intact form.Once these non-digestible carbohydrates pass into the intestines, they serve as a feast for the probiotic bacteria that live there.

Prebiotics of proven efficacy are able to modulate the gut microbiota by stimulating indigenous beneficial flora while inhibiting the growth of pathogenic bacteria therein. Preferred target organisms for prebiotics are species belonging to the Lactobacillus and

Bifidobacteriumgenera.The most common prebiotics are oligosaccharides, which are found in human milk.

For the food ingredient to be classified as a prebiotic, the following three criteria have been defined:

- The food ingredient must not be hydrolyzed or absorbed in the stomach or small intestine
- It must be selective for beneficial commensal bacteria in the colon by encouraging the growth/metabolism of the organisms;
- It will alter the microflora to a healthy composition by inducing beneficial luminal/systemic effects within the host.

Any food substrate that enters the colon may be a potential prebiotic, however, selective fermentation is a necessary determinant.Much of the early and present work on prebiotics has been carried out in Japan. The search for bifidobacteria promoting substances began by screening a range of carbon sources for their ability to increase these organisms in pure culture.

For example, Yazawaet al. (1978) screened a range of dietary carbohydrates for their ability to promote bifidobacteria in comparison to other intestinal isolates.[67]Further studies used mixed culture, animal models and human trials to determine the efficacy of oligosaccharides to modulate the gut flora composition.

Synbiotic-

Symbiotic is a product that contains both probiotics and prebiotics.It is nothing but the synergy between probiotic and prebiotic effect in the GI tract or in other words, synbiotic is the usage of both probiotics and prebiotics in combinations.Indeed synbiotic combinations are considered to have more beneficial effects on human health than probiotics or prebiotics alone.

Recent studies established that synbiotics improve the intestinal microbial environment and activate host immune function, leading to prevention of bacterial translocation.

Different studies regarding probiotic and prebiotic usage in neonates and their results.

Deshpande G, Rao S conducted a study called Updated metanalysis of probiotics for preventing necrotizing enterocolitis in preterm neonates in the year of 2010 showing the result that risk for NEC and death was significantly lower in prebiotic and probiotic group. Risk for sepsis did not differ significantly. No significant adverse effects were reported. Trial sequential analysis showed 30% reduction in the incidence of NEC.

Suzanne M Garland, Jacinta M Tobin conducted a study multi-center, prospective, randomized, double blind, placebo controlled trial investigating the treatment of very preterm infants with a probiotic combination comprising Bifidobacteriuminfantis, Streptococcus thermophilusandBifidobacteriumlactis In the year 2011 a total of 1100 subjects were included in the study in different centers of Australia and new Zealand showing results from previous studies on the use of probiotics to prevent diseases in preterm infants are promising. However, a large clinical trial is required to address outstanding issues regarding safety and efficacy in this vulnerable population.

Carole Rouge´, HuguesPiloquet, conducted a study in the year of 2009 , Oral supplementation with probiotics in very-low-birth-weight preterm infants: a randomized, double-blind, placebo controlled trial supplementation with Bifidobacteriumlongum& Lactobacillus rhamnosus GG (BB536-LGG) may not improve the gastrointestinal tolerance to enteral feeding in very-low-birthweight infants but may improve gastrointestinal tolerance in infants weighing >1000 g.

Taciana Duque Braga, GiseliaAlves Pontes da Silva, conducted astudy in 2011 A double-blind, randomized, controlled clinical trial was conducted in 231 preterm infants weighing from 750 to 1499 g at birth showed that oral supplementation of B. breveand L. caseireduced the occurrence of NEC (Bell's stage >2).

Mary N Mugambi , Alfred Musekiwa, conducted a study in the year 2012 named Probiotics, prebiotics infant formula use in preterm or low birth weight infants concluded that there is not enough evidence to

suggest that supplementation with probiotics or prebiotics results in improved growth and clinical outcomes in exclusively formula fed preterm infants.

Susan E et alconducted a study between 2007 to 2011. A total 1099 very preterm Infants from Australia and New Zealand were randomized Rates of definite late-onset sepsis (16.2%), NEC of Bell stage 2 or more (4.4%), and mortality (5.1%) were low in controls, with high breast milk feeding rates (96.9%).No significant difference in definite late-onset sepsis or all-cause mortality was found, but this probiotic combination(B infantis, S thermophilus, and B lactis) reduced NEC of Bell stage 2 or more (2.0% versus 4.4%; relative risk 0.46, 95% confidence interval 0.23 to 0.93, P = .03;

Mihatsch, Vossbeck,Eikmanns,A Randomized Controlled Trial Effect of Bifidobacteriumlactison the incidence of nosocomial infections in Very-Low-Birth-Weight Infants.There were 93 infants in the B. lactisgroup and 90 in the placebo group,there was no significant difference between the two groups with regard to the incidence density of nosocomial infections (0.021 vs. 0.016; p = 0.9, x 2 test).There were 2 cases of NEC in the B. lactisgroup and 4 in the placebo group concluded that in the present setting, B.lactisat a dosage of 6 ! 2.0 ! 10 9 CFU/kg/day (12 billionCFU/kg/day) did not reduce the incidence density of nosocomial linfections in VLBW infants. No adverse effect of B. lactis.

Hung-Chih Lin, Bai-HorngSu, An-Chyi Chen conducted a study in 2005 a randomized control trial was conducted to evaluate the beneficial effects of probiotics in reducing the incidence and severity of NEC among VLBW (<1500 g) infant. The strains used in the study (Lactobacillus acidophilus and Bifidobacteriuminfantis). A total of 367 infants were enrolled in the study 180 in the study group and 187 in the control group,the incidence of death or NEC (≥ stage 2) was significantly lower in the study group (9 of 180 vs 24 of 187), the incidence of NEC (≥ stage 2) was also significantly lower in the study when compared with the control group (2 of 180 vs 10 of 187)and there were 6 cases of severe NEC (Bell stage 3) in the control group and none in the study group. None of the positive blood culture grew Lactobacillus or Bifidobacterium species.The overall results showed that probiotics fed enterally with breast milk reduce the incidence and severity of NEC in

VLBW infants.

Studies related to prebiotic and probiotic supplementation in preterm infants in related to feed intolerance duration of NICU stay, mean weight gain and mortality -

Carole Rouge´, HuguesPiloquet,Marie-Jose´ Butelconducted a study in preterm infants in 2009 supplementation with Bifidobacteriumlongum, Lactobacillus rhamnosus GG may not improve the gastrointestinal tolerance to enteral feeding in extremely -low-birthweight infants but may improve gastrointestinal tolerance in infants weighing >1000 g.

Sari FN et al.in the year of 2010 total to 221 babies enrolled in the study group, and this study showed that feed intolerance was significantly lower in the pre-and probiotic group as compared to control group but single strain (L.sporogens)) probiotic was used in this study as compared to our study we have used mixture of multiple strain or organism..

Rojas MA et al in 2009 also found that duration of NICU stay was less in probiotic group than the placebo (p-0.04).

Moni S.C. et all.conducted a study in 2015 also showed that the duration of stay was less in probiotic group compared to placebo group which was statistically significant.

Susan E et al in 2013 conducted a study trial in that he did not find any decreased or reduction in mortality in probiotic group as compared to placebo.

Wang Q et alin his trial of probiotic in preterm babies and NEC shows that mortality rate was less in preterm VLBW babies treated with pre and probiotics compared with placebo group

Yang Y. et al as shown that two trials with 205 babies in cases and 199 baby is in control group, studied weight gain in this group and they didn't found any statistical difference between the two groups but there was a significant heterogeneity among these two trials.

Moni S.C et al. in the year of 2015 conducted trial with s 65 preterm infants and found that mean weight gain was good in probiotic group which was statistically significant(p-0.000).

3 CONCLUSION

Premature infants, especially very low birth weight (VLBW), are at risk of neonatal morbidity and mortality. Immaturity of the organ systems of preterm infants makes them more susceptible to many complications including respiratory distress syndrome, bronchopulmonary dysplasia, necrotizing enterocolitis (NEC), and patent ductusarteriosus, sepsis, and anemia, retinopathy of prematurity and intraventricularhemorrhage.

To survive the extra uterine environment, preterm infants often require special care and extensive support in the neonatal intensive care unit.

Aside from its digestive and absorptive functions, the gastrointestinal (GI) tract is an essential immune organ and the largest defense barrier protecting the host from pathogens, toxins and subsequent inflammation while allowing commensal bacteria to grow.

The micro biome is a complex ecosystem consisting of more than 1000 species of live bacteria that play major roles in nutrition and in the development of the immune system. The combination of probiotics and prebiotics (synbiotics), which may be as important as probiotics in improving premature infant gut's health.

The development of the neonatal micro biome begins with the exposure of the fetus to microbes in the amniotic fluidand continues to diversify depending on factors such as GA, mode of delivery, hospitalization, antibiotic use and type of feeding. Unlike the micro biome of the term infant, the preterm infant micro biome is less diverse and is predominated by Staphylococcus species, with Bifidobacteriumspecies being less well represented. To conclude probiotics and prebiotics appear to be useful in pre term babies with NEC and feed intolerance.

4

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