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Microbiome And Microbiota - An Influencer Early In TheLife.

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Abstract:
Preterm delivery increases morbidity and mortality in neonates owing to its numerous riskfactorsassociatedwithprematurity.Inuterofetuscontinuallyswallowsamnioticfluidthat plays an important role in growth and development of most vital organs as Lungs, Gastro IntestinalTract(GIT)andalsoformsamajorpartofmeconium.Oncethoughtsterileamniotic fluid is now recognized to harbor numerous microbiome that are considered essential in development of immunity and neurological function [1]. Human development and physiology are largely determined based on this microbiome. Bacterial cells outnumber native epithelial cells providing certain beneficial effectsin the body. Ideally intestine protects the host from pathogens and toxins as that remain as a largest interface between host and external environment and allow colonization of commensal harmonious bacteria.Delayedintroductionofhumanmilkorprolongedfastinginneonatesmayalternormal gastrointestinal (GI) functions that are stimulated by human milk such as GI motility and various hormonal secretions. Minimal enteral nutrition or trophic feeds are initiated at 15 – 20 ml/kg/day every 2-3 hours and are continued without increasing for 5 – 7 days [2]. Usually Extremely Low Birth Weight (ELBW) infants or preterm neonates develop Necrotizing Enterocolitis(NEC)at2 to 3 weeks of life.NECisacommonGIemergencyinneonatesandleading cause of mortality and morbidity in approximately 1 in 10 infants born at less than 29 weeks gestation and 20 – 30 % of infants < 1500 gm die [3]. Human breast milk has an osmolarityof300 mOsmol/Kg in addition fortification with sodium and other supplementations can contributetoexcessosmolarityupto400mOsmol/Kg.Increasedosmolarityinintestinallumen would result in mucosal damage. Bifidobacteria, Proteobacteria and Bacteriodespredominating the neonatal gut with less Firmicutes were proven beneficial.[2]. Those beneficial microbes secrete chemical ligands that react through Toll Like Receptors (TLR) which are unique to the microbes and co-ordinate with innate immune system. Intestinal integrity an essential factor to prevent NEC is largely based on these interactions. As these receptors are needed for production of recovery proteins as heat shock proteins and Tumor Necrosis Factor (TNF) they are beneficial in developing immunity [4]. Commensal beneficial bacteria secrete TLRligandsaslipopolysaccharideandlipoteichoicacidthatinteractwithnormalintestineand enhance the ability to withstand injury and prime the cells for repair. Variation in gestational age, weight, mode of delivery and exposure to antibiotics are shown to alter the native gut microbiome drastically and alterations in them affects immune and neuronal development[5].

Keywords:*Microbiota, probiotics, NEC, Human Milk Oligosaccharides, Bifidobacteria*

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I. Introduction:

Microbiome influence the actions of immunologic, endocrine and neural pathways, majority of which flourish in the intestine. Gut microbiota plays essential roles including protective mechanism against pathogenic

bacteria, digestionof colostrum, breast milk or any otheralternative feedings,breakdownof toxins,vitaminsynthesis,ironabsorption,growth&differentiation of epithelial lining of the intestine, maintaining homeostasis of immune system and tolerance to food antigen. [5,6]. Immune homeostasis and complete gut colonization happen at 3 years of age. Inappropriate colonization may lead to dysbiosis which increase susceptibility to variety of immune related pathogenic state or adverse immune outcomes[7].Microbiotaprovidinganimportantbarrierbetweenhostandenvironmentininfant GI depends on original inoculum, immediate living environment, feeding practices. This barrier helps facilitating pre-digestion of many nutrients, prevents settlement of unwanted or pathogenic microorganism providing mutual support to gut microbiota and micro ecological environment [8,9]. Early and immature microbiota is unique to every human being that diversifies and stabilizes with breast feeding and weaning. Human microbiota plays an important role in nutrition by providing energy three metabolic process from many nutrients. Sequencing human genome and microbial genome have provide an idea about intestinal microbiota and individual health [10]. Probiotics demonstrate positive effect on health by providing live microbial food supplements [11]. It alters the development and composition of intestinal microbiota and their activity. Probiotics facilitate cross-talk with immune system and microbes through direct contact with mucosal cells[12].

Molecular studies indicated that gut microbiota is characterized by early colonization with facultative anaerobes as *Enterobacter, Lactobacilli* and *Coliforms* which is rapidly succeeded by anaerobic genera as *Bifidobacterium*, *Bacteroides*, *Clostridium* [13]. About 60 – 90 % of breast-fed infants fecal microbiota is composed of *Bifidobacterium* and lactic acid producing bacteriaaccountfor<1%whereasinalternativefedinfantsthecompositionismorecomplexand depends of the type of feeding[13, 14]. The variation occurs in number as well as in species, breast fed infants have more of *Bifidobacterium breve*, *B. infantis*, *B. longum* and other types innon-breast-fedinfants.Thisvariationisduetosupplyofoligosaccharide–afavorablesubstrate for *Bifidobacterium* and also some bifidogenic effect provided by maternal skin and Breast Milk [2]. Host microbe cross talk maintains peaceful co-existence and mutual benefit between organism of 21 more different species and their benefits from association. *Bacteroides* are shown to modulate glycosylation of mucus and induce production of antimicrobials thus shapingthe immune system. They also evade detection by immune system by changing capsule polysaccharidecompositionandsurfaceantigenicity which helps theseoriginalcolonizerstostayinthe gut for long term [7]. GI epithelium equipped with pattern recognition receptor including Toll Like Receptors recognize specific conserve pathogen associated molecules patterns, but distinguishing between pathogenic and non-pathogenic bacteria is difficult by TLRs [15]. Signaling pathway of TLRs results in production of pro-inflammatory cytokines through activationoftranscriptionNuclearFactorkβ(Nkβ).HealthygutmicrobiotaactivatesTLRand the resulting activation produces some anti-inflammatory mediators in gut [16]. Barrier in the intestinalmucosaproduceabundantIgAthathelpseliminatingtheantigens[17].Otheradaptive immune response stimulated by cytokines and chemokines have helped preventing the commensalfrombreachingthegutwallaftermaturationofdendriticcells,whereaspathogenic bacteria destroy it [18]. Communication of gut microbiome with host immunity is important in neonatalperiodasthecommensalbacteriaprovideantigenchallengeandstimulatematuration of gut associated lymphoid tissue so probiotics indeed provide impacts beyond just nutrition [12]. There are 10^9 live bacteria per liter of human milk and it is a source of *Staphylococci*, *Streptococci*,Lacticacidbacteriaand*Bifidobacteria*.Amongbreastfedinfants*Bifidobacteria*, specifically*B.longum*,*B.infantis*and*B.breve*are predominant reachingupto60–90%oftotalfecalmicrobiota.[10, 19]

Bidirectional communication of intestinal microbiota and host immunity:

InfantsreceiveIgGandIgAfromplacentaandbreastmilk.HabitatsofGItractsdepend on presence of IgA specific for intestinal bacteria. IgA bound bacteria colonize effectively in the small intestine and those that not bound to IgA colonize large intestine [20]. Segmented Filamentous Bacteria colonize the GI tracts and induce immunity by promoting IgA secretion and development of intestinal cell lymphocytes. T Helper cells play an important role in stimulating cytokine production. Th17 cells play a key role in amplifying andstimulatingcytokinesIL–17,IL-22andIL–17Fabundantlyinthemucosa,therebyimproving the barrier function of GIT. Human GIT has several hundred bacterial species in mutualistic relationshipwithhostandimmunesystem[16,18].Ahealthygutmicrobiotaservesvarioususeful function as digestion

of polysaccharide, degrading toxic products and barriers for pathogenic bacteria and fungi [21]. Dysbiosis of the gut microbiome alter the symbiotic relationship and is associated with various diseases as Inflammatory Bowel Disease, obesity, allergy and autoimmune and neurological disorder[22].

Breast milk microbiota and Beneficial factors:

WHO recommends exclusive breast feeding to all infants up to 6 months of age and continued supplementing Breast Feeding up to 24 months of age and beyond. Breast milk is considered as most beneficial and nutritious food for newborn and its contents tend to vary with infant's requirement according to the growth [19, 23]. Recent study show that breast milk is not sterile havingabout600differenttypesofbacterialspecieswith10^3to10^4CFU/mlofbacterialcells [22, 24]. Lactic acid bacteria and *Bifidobacteria*are the most commonly isolated species from breast milk [1, 25]. There are certain other bacteria *Veillonella, Leptotrichia and Prevotella*that inhabitant the oral cavity [5, 16]. TGF – β levels in breast milk are associated with fatty acids as theyarepositivelycorrelatedwithPUFAandnegativelywithsaturatedFattyacidcontent.(24) Breast Milk also serve as a source of Prebiotic (Human Milk Oligosaccharides), Lactoferrin (an antimicrobial protein) and Lysozyme (Bactericidal enzyme) [20]. Overall mothers milk plays a vital role in gut microbial composition with induvial variability in oligosaccharides that are unique. Oligosaccharides - a 2 – 20 repeated sugar units with a beta glycosidic bond and is resistant to degradation by human GIT which lacks enzymes to degrade. Once in the intestine oligosaccharides can serve as probiotic to beneficial bacteria [9, 22]. Human Milk Oligosaccharides (HMO) are difficult to produce, so commercially available Plant based Oligosaccharides such as Fructo-oligosaccharides (FOS) and Galacto-oligosaccharides (GOS) are used as alternatives which shown to increase *Bifidobacterium* and also decreased incidence of gastroenteritis and respiratory disorders[9,21].*Bifidus*bacteriumfermentonthepartiallydigested GOSbytheenzymeLactose-n-biosidase in the small intestine and produce short chain fatty acids (SCFAs) [20, 22]. As there are more *Bifidobacterium* in the intestine the amount of HMOs in the feces decreases and the amount of acetic and lactic acid levels are increased [22]. Lactose is a prebiotic enhancing the growth of beneficial microbes such as *Bifidobacterium* [10, 17]. *Lactobacillus* binds to epithelial cell and inhibit pathogenic bacteria in GIT of neonates by providing anti-microbial effect and also withthehelpoflacticacidproductionandloweringthepH.ItalsolowersthepHbyproducing lactic acid, killing pathogenic bacteria. Colonization of commensal bacteria will prevent pathogenic bacteria from colonizing the gut [9]. Probiotic supplementation enhances the colonization of beneficial bacteria especially in preterm infants. Lactobacillus is increased in probiotic supplemented premies, compared with preterm who were on antibiotics, alternativefeed or very low birth weight infants [2]. Oligosaccharides in breast milk increased colonization of BifidobacteriumandNECrateswerehigherininfantswhowereonacidsuppressivetherapy[14, 25]

Maternal nutrition and gut microbiota in neonates:

From gestation to lactation – Mother deliver microbiota to the infants in a healthy and equilibrated pattern. Perinatal nutrition and microbial environment can cause permanent modification in fetal physiology. Care before conception to the women of child bearing age or desire to get pregnant or regardless of pregnancy status should be provided with proper and balanced nutrition. Under nourished women could negatively affect the fetal growth, immunologicalandnutritionalstatusoftheinfant.So,fortifyingthefoodsandrationalizingthe nutritional needs of pregnant women with micro and macro nutrient including Iron, Vitamin A, Folic Acid, Iodine etc is considered essential [10]. Nutritional status in a pregnant woman can be assessed by measuring weight, height and BMI from early weeks of pregnancy. Maternal complications like Pre-eclampsia, Type 2 Diabetes mellitus during pregnancy are found to be connected with rise in BMI. When a mother is undernourished, the fetus does not getenoughnutrients and presentseitheraslowbirthweightorpoorgrowthratewhichcoulddamage immune system increasing the risk to develop a disease [26]. Supplementation of polyunsaturated fatty acid (PUFA) is highly recommended during the last trimester of pregnancy as this is considered as the period of brain growth and highest DHA accumulation in neural tissue tend to happen during this phase of fetal growth. Any alterations in DHA levels could present with cognitive impairment, developmental delay and reduction in the size of the brain. Major nutrient of human milk is lipid, and they are directly related to brain development. Docosahexaenoic Acid (DHA) and PUFA content in milk were influenced by mother's diet and it is in higher side in women who consume fish on regular basis. This is based on evidence that Omega 3 fatty acids demonstrate beneficial effects

on cognitive development and mental development of children. Maternal microbiota is largely based on biochemical parameters which include increased folic acid and ferritin levels and decreased transferrin and cholesterol levels which favors *Bifidobacterium* group [22,27].

II. Critical Window Of Development OfMicrobiome

There are 10^{14} bacterial cells which outnumber native human cells. Initially aerobic environment in the infant gut which later gets anaerobic due to altered colonization [1, 3]. The colonization in first few days of life is dynamic, simple and very unstable as it undergoes alterations which influences intestinal morphology, immune system development and Gut Associated Lymphoid Tissue (GALT) function [9, 20]. Intestinal cells detect and discriminate between self-antigens, food antigens and normal commensals from pathogenic bacteria.Immature immune system of premature GI mucosa will predispose infant to hyper inflammatory response for microbial stimuli. Two big transitions occur in infancy during the process of stabilizing the gut microbiome, first transition occurs soon after birth, during lactation dominated by *Bifidobacterium*. Second transition occurs during weaning period with breast feeding and introduction of solid foods resulting in establishment of adult like microbiomedominatedwith*Bacteroides*and*Firmicutes*[5,22].Enterotypesknownasstablegut microbiota dominated by *Bacteroides, Prevotella*and *Firmicutes* maintain well balanced host microbiotasymbioticstates[28].Intestinalbacteriaanditsmetabolitesasshortchainfattyacids helps maintaining the proliferation and differentiation of regulatory and helper T Cells and B CellsasimmunoglobulinsIgAandIgG[22].Meconiummicrobiota is classifiedintotwotypesthe first less diverse, dominated by *Enterobacteriaceae* and the later more diverse, dominated by *Firmicutes* mostly Lactic Acid Bacteria [1]. As the baby is bathed in amniotic fluid, the fetus GI tract is colonized by bacteria similar to amniotic fluid, they resemble microbiota of amniotic fluid suggesting microbes in meconium originate from uterus of the mother. Infant microbiota change during first year of life as the microbiome initially were enriched with genes facilitating lactate utilization whereas in the later part of weaning,they're enriched with genes that facilitate carbohydrate utilization [22,25]. Theinteractionbetweenbacteriaandhumanhostisconsideredmutualisticandtheyprovide metabolic,trophicorprotectivefunctions.Variousfactorsinfluencinggutmicrobiotaofinfants upto24monthsofageareGestationalage(Term/ Preterm),NormalVaginalDelivery(NVD) / Lower Segment Caesarian Section (LSCS), maternal weight and diet, use of antibiotics in early days of life, breast Feeding / Alternative Feed, use of Probiotics supplemented feeds and food chosen for the infant and the time of weaning from breast milk [1,9].

VLBW infant Gut microbiome: growth, immunity anddevelopment

Human milk provides nutrients for bacterial growth (HMOs) that could ultimately help in differentiating epithelium, GALT, GI morphology, nutrition and metabolism [9, 20]. Preterm gut microbiota is less diverse than full term infants and greater risk for dysbiosis due to immature immune system and physiology. Healthy colonization called eubiosis – dominated by gram positive *Propionibacterium, Bifidobacterium, Lactobacilli* which have the capacity to digest HMO are beneficial [2]. VLBW infant develop a sparce microbiome and they are more CONS, *Enterococci* and *Enterobacteriaeceae*withverylessanaerobes.Pretermverylowbirthweightinfantsaremostly exposedtomultipleprenatalandpostnatalinsultsduringrapidC-Sectionorvaginaldelivery reducing exposure [24]. They can also be exposed to many factors that affect prenatal maternal illness, as chorioamnionitis, vaginal infections or physiological stress. VLBW neonates requiring prolonged hospitalization are more likely exposed to a microbiome that is very different from normal infant, usually with Intensive Care Unit flora [21, 29, 30]. Apart from the various factors thataltermicrobiome,nutritionandcertainmedicationusedalsoaltersthediversityoftheflora. *Actinobacteria* and *Proteobacteria* are associated with lipid, *Firmicutes* with protein and all three has common association with carbohydrates. Eubiosis is achieved with increased abundance of *Bifidobacterium* and *Actinobacterium. Bifidobacterium* association was increased with lipid in diet and certain medications as corticosteroids and H2 receptor antagonists [5].

Lipopolysaccharides (LPS) in the cord blood of preterm infants has evidenced the translocation of microbiome and changed the thought of being essentially sterile to the confirmatory presence of microbial life in infants [1, 2, 8]. Usually, it is anaerobes in colon and distalsmallintestineandfacultativeanaerobes,aerobescolonizetheupperGIT[1,5].Asthegut of VLBW infant is immature and fragile, the feeding patterns of them were entirely different from that of normal neonate. Dysbiosis in VLBW infants could increase the risk of sepsis and NECasthesethreatscouldhappenanytimeduringtheirhospitalization[27].Thecauseofthese could be secondary to immature immune system interrupting the enterocyte to control invasive pathogenic bacteria and set succession for inflammation and necrosis that is characteristics of NEC remains the major cause. The invasion is easily demised by Secretory immunoglobulin (IgA) in milk, which takes charge in exclusively breast-fed infants [31]. Immune system of an infant is established based on commensal microbiota as remarkable interactionshappensinchildhoodasearlygutrespondsdifferentlytoinflammatorystimuli[23]. Development of innate and acquired immune system of an infant requires co interaction of developing gut microbiome with host immunity[22].

III. Brain Gut Enteric MicrobiotaAxis:

Gut brain communication.

Critical window during gut priming by human milk that provide HMO for beneficial microbiota is essential for neurocognitive and emotional development. Maternal prenatal stress influences the HPA axis and the infant microbiome has positive association with pathogenic bacteria and inverse associationwiththebeneficialbacteria.Thealterationingutmicrobiomeisrelatedtoincrease production of IL-6 that is associated with developing brain, interfering with white matter and brainplasticity.Itmakesessentialtomaintaininfant'smicrobiomeduringfirst1000daysoflife [24]. Goblet cells that remain as specific biological machinery in secreting mucus is present throughoutthelengthofintestine.Intestinalepithelialcellsareproducedfrommultipotentstem cells residing at the base of intestinal crypts which then migrate to villus just prior shedding into the lumen. This production and maturation of GI tract stem cells involves enteric nervous system but the morphology keeps changing through cellular life span. Microbial population is distributed throughout the GIT from luminal to mucosal axis. Viscosity of mucus increases towards the distal region of GIT and that viscosity gradient determines the distribution of intestinal microbiota. The variation in oxygen levels and nutrient availability also alters the mucosal to luminal bacterial distribution. These microbes produce mucus degrading enzymes as glycosidase, sialidase and sulphatase which cleaves the glycans to monosaccharides that is laterutilizedbymucusresidingbacteria.Thephysicalpropertiesofmucuslayeraredependent on these symbiotic, mutualistic interactions ofmicrobes.

Digestive system is innervated by intrinsic neuronal network regulating the GI functions. Twoganglionicplexusregulatesneuronalcontrolofintestinalfunctions,asthemyentricplexus that maintains GI motility and the Submucosal plexus – Maintains secretion of water and electrolytes with the help of some secretagogues like acetylcholine and Vasoactive Intestinal Peptides (VIP). Microbiome in intestine regulates host metabolic pathways that pave host- microbiota signaling, immune and inflammatory processes in intestine, liver, muscles and brain. Vagus nerve that innervates the gut helps maintaining homeostasis and influence behavior directly through interacting with Hypothalamic Pituitary Axis (HPA), peptide produced by gut wall as a resulting interaction of microbiome to the gut wall stimulate afferent ending of vagus nerve transmitting to CNS affecting neuralactivity and behavior[7].

Immune and endocrine Pathways

CommensalcolonizationisessentialforthepropердevelopmentandgrowthofGItract vascularization and barrier function. The major barrier functions in GI are tight junctions between epithelial cells and mucous layer covering epithelial lining. In preterm neonates the defense mechanisms are immature allowing adherence and penetrance of bacteria and toxic substrate. Probiotics and lactobacillus help in maintaining the tight junction between epithelial cells and increase production of mucus preventing bacterial adherence and increase clearance respectively [18]. Probiotics also helps in attenuating the epithelial apoptosis that is more pronounced in preterm. Commensal bacteria in gut can enhance the GI mobility in turnhasten the elimination of potential toxins or pathogens [25]. Hypothalamic Pituitary axis (HPA) influences cortisol secretion affecting stress response [22]. Gut microbiome induced pro-inflammatorycytokinesandincreasedcortisolsecretiontogetheraugmentsthegutpermeability enhancing the LPS

leakage across the gut wall [2]. Stress induced alteration in gut microbiome is shown to decrease *Lactobacillus* related to increased production of IL-6 and anxiety. This breach of gut wall integrity stimulates robust inflammatory response happening mostly with GNB like *Enterobacteriaeceae*and *Pseudomonaeceae*[7]. Lactobacillus is protective bacteria to gut by lowering the pH prohibiting colonization of more inflammatory and pathogenic microbes. Colonization of *Staphylococcus* has elevated levels of cytokines suggesting early dysbiosis impacting the development of immune system[1].

IV. Dysbiosis And Development Of Nec:

Pre-natal:

PCR studies of amniotic fluid estimated the prevalence of certain culture resistant anaerobic like *Leptotrichiaspp*and *Sneathiasanguinegens*belonging to *Fusobacteriaceae*in abundance. The evidence for this colonization affecting the neonatal gut is not clear but the factthatneonateisbathedinamnioticfluidinutero,soisthegastrointestinaltract.Duringthe last trimester as the fetus swallow large amount of amniotic fluid and recent findings of microbial DNA in meconium correlates to the above proposedhypothesis[1]

Post-natal: Various factors contribute the postnatal bacterial colonization as,

1. NormalVaginalDelivery(NVD)VsLowerSegmentCesareanSection(LSCS):

Babies bornwithC-Sectiondeliveryhadpotentiallypathogenicbacteriaas*Staphylococcussps*and *Acinetobacter spstypically* found in skin surface and hospital compared to babies born in Normal Vaginal Delivery (NVD) who are colonized mainly with *Lactobacillus Sps*[3, 29].Through vertical transmission the infant microbiome in NVD will be predominant with mother vaginal and fecal flora with more of *Lactobacillus*, *Prevotella*and had dominance of *Bifidobacterium* and *Bacteroides* within months, whereas an infant born by cesarean section has bacterial flora on skin surface dominated by *Staphylococcus*, *Corynebacterium*and *Propionibacterium* and lesser *Bifidobacterium* and *Bacteroides* [14]. *Lactobacilli* protect against pathogenic bacteria by producing lactic acid and other antimicrobial substances. Decreased *Lactobacilli* is also a risk factor for PROM. Preterm birth and perinatalcomplicationsincreasedwithbacterialvaginosisanddecreasedlevelsofhydrogen peroxide producing *Lactobacillus* [2]. The diversity of bacteria provides more protection in the gut and lack of its cause more human diseases [14, 29].

2. Types of feeds:

Breast milk has multiple nutritional components with other non- nutritional natural immunoglobulins (IgA), lactoferrins, cytokines, growth factors, Poly Unsaturated Fatty Acids (PUFA), Platelet Activating Factors (PAF) acetyl hydrolase. Although commercially available alternatives provide energy requirement similar to breast milk, they lack non-nutritional parameters that are essential for immunity and development in neonates. The non-nutritional factors contribute to mucosal integrity and function, boost immunity against various GI infection [27]. Many commensal bacteria that are promoted by Human Milk Oligosaccharides (HMO) containing lactose core[29].Newborngutgetcolonizedwith variety with microbial population in the first year of life and that new born diet represent an essential extrinsic factor in colonizing the gut. Breast fed infants dominated with *Bifidobacterium*, few anaerobes because of the existence of HMOs. As infants have shortfall of enzymes that digest HMO they are eventually passed over to intestine where they act as prebiotic and augment the growth of *Bifidobacteria*and *Bacteroides*, whereas infants fed with alternate feeds have predominance of *Escherichia coli, Clostridium difficle*and *Bacteroides fragilis* which could subsequently develop atopy[7].

3. Exposure to antibiotics:

Pre-Probiotics are essential factors in modulating the infants gut microbiota but antibiotics used in early stages of life could be detrimental [9]. Rapid colonization of intestinal bacteria happens soon after birth in newborns with facultative anaerobic or aerobic bacteria as *Enterobacteriaceae*, *Enterococci* and *Staphylococci.* During growth process they consume oxygen and let the anaerobic bacteria as *Bacterioides*, *Bifidobacterias*and *Clostridia* to proliferate [14, 19]. This transition tends to nothappeninalternativelyfedinfantandpatternofcolonizationvarieswithrespecttobreastfed infants predominating with gram negatives and few anaerobes. Intrapartum antibiotics reduces beneficial bacteria in infants and also delays the colonization of commensal bacteria. Using antibiotics in the mother or prophylaxis antibiotics in preterm neonates could affect the microbial diversity and provides a window of opportunity for pathogenesis

and antibiotic resistant bacteria to grow leading to diseased state[29].

4. Gestational Age:

Variation in microbial composition directly or indirectly alters the energy harvest and storage affecting the weight gain of the preterm neonates. As there are variations in preterm and term neonates, the vaginally delivered and exclusively breastfed infant is considered as gold standard for healthy infant microbiota. The gut microbiota of preterm infants is characterized by delayed colonization of beneficial bacteria also less diversity. Gestation age is proportional to the colonization of obligate anaerobic bacteria and inversely related to facultative anaerobes that are considered pathogenic. Organisms such as *Enterobacter, Enterococcus, Escherichia* and *Klebsiella* are seen in preterm than in term neonates. There are other factors that influence the gut microbiota of preterm infants from term neonate due to its indispensable and unique set of environmental conditions including hospital environment of Neonatal Intensive Care Units [(14)].

V. NEC And Alteration Of Intestinal Microbiota:

The manifestation of vascular, mucosal and metabolic insult to immature intestine of neonates, NEC is a common gastrointestinal emergency and one of the leading causes of morbidity and mortality in NICU [35]. The risk factors associated with NEC include prematurity, low birth weight, asphyxia, acute cardiopulmonary disease, polycythemia, hyper viscositysyndrome,exchangetransfusion,aggressiveeternalfeedingorabnormalcolonization with pathogens. Abdominal tenderness or distension, feeding intolerance, bloody stools and apnea are the suspicious first signs of NEC [14]. Definitive signs include pneumatosis intestinalis, portal venous gas in abdominal x-rays, other routines like CBC with differentials.To better classify and diagnose NEC, Bell's staging criteria were developed. As per Bell's stagingcriteriasuspectedNECisclassifiedasStage1,ProvenNECasStage2andseverecases as Stage 3. Although there is medical treatment for NEC, there are few approaches to prevent NEC such as every breast-feeding initiating tropic feeding to prevent gut atrophy, intestinal colonizationwithcommensalbacteriatomaintainintegrityofgastrointestinalmucosalbarrier[32, 33].

Toll like receptor - a receptor for lipopolysaccharide bacterial outer membrane component upon stimulation result in activation of transcription Nuclear Factor Kappa β (NF-kβ) which then trigger synthesis. Toll like Receptors (TLR) a Pattern Recognition Receptors (PRR) havingtheabilitytorecognizeMembraneAssociatedMolecularProteins(MAMP)withunique TLRs [7, 16, 17]. Activating specific TLRs as in TLR2 and TLR4 by gram positive and gram- negative bacteria with peptidoglycans and lipopolysaccharides respectively lead to activation of NK-kβ and caspases which induces cytokines as IL1, IL6, IL8, TNFα and INF-1. Healthy and beneficial commensals are not shown to induce inflammatory mediators instead they tend to protect the intestinal mucosal cells [25,35]. Secondary to abnormal colonization, bacterial translocation occurs which induces bacterial toxins or antigens that damage the intestinal epithelium that can also enter the circulation resulting in inflammatory responses. Nitricoxide thatisexpressedasaresultofinflammationdisruptthetightjunctionzonulinproteinsandoccludin, once disrupted the damaged mucosal barrier permits increased permeability and microbial dislocation [14,35]. Gamma proteobacteria colonization happens showing early dysbiosisprecedingNECinmanyPreterminfants.Alterationinmucosalbloodflowinpreterm due to varied prostaglandins, nitric oxide and epidermal growth factor influences mucosal blood flow, oxygenation and mucosal integrity, Probiotics enhances the intestinal blood flow and theorized to be a result of production of vasoactive amines[25].

VI. Oral Probiotics And NEC:

Probiotics is derived from Latin and Greek means for living and defined by Food and Agricultural Organization (AFO) by 1965 as "live microorganism which, when consumed in adequate amount as part of food, confer health benefit on the host [3, 9, 33]. Three decades later in 1995, Gibson and Roberfroid defined prebiotics as a non-digestible food ingredient that affect the host by selectively stimulating the growth and / or limited number of bacteria in the colon improving the health [9]. Probiotics in the gut upregulate cytoprotective gene, downregulate expression of pro inflammatory gene expression, produce SCFA and butyrate helping the colonocyte and also reduce pH, barrier maturation & function, regulations of cellularimmunityandTh1&Th2balance[14,35].Oncesuspectedfeedingshouldbewithdrawn, initial management involve bowel rest, decompression obtain blood culture before

administeringbroadspectrumantibiotics,etc.adequatefluidresuscitationandserialabdominal radiographs essential for surgical interventions in severe cases[3].

Probiotics competes with other pathogenic bacteria for binding sites and substrates in bowelthatenhanceIgAmucosalresponseandimprovethebarrierthatcouldeffectivelyreduce permeability and increase anti-inflammatory cytokines and stimulates mucosal lactaseactivity and production of antimicrobial substates as bacteriocins, microcins, hydrogen ions, and Hydrogen Peroxide [8, 18]. The largest immune organ - intestine provides the immunological barrierbyenablingthecrosstalkbetweenmicroorganismandintestinalepitheliumwhichact as a second barrier function. This microbial host interaction provides benefits by various mechanisms promoting the mucosal integrity, reducing inflammation, activating intestinal immune defenses and regulating appropriate colonization[17].At cellular level probiotics attenuate NF-kβ activation which is a major proinflammatorypathway,preventcelldeathandapoptosiswithupregulationofcytoprotective genes,generatesreactiveoxygenspeciesessentialincellsignalingandincreasedinductionand expressionoftightjunctionprotectionessentialforbarrierfunction.Becausepretermguthas delayed colonization and minimal bacterial diversity, it is also easily amenable to manipulate. Soearlyadministrationofbeneficialprobioticsas*Lactobacillus*and*Bifidobacterium*isproven beneficial. Another approach towards prevention of NEC would be prebiotic administration [36].Prebioticsarenon-digestibledietaryproductsimprovethehealthofintestinebypromoting selecting growth of beneficial bacteria [33]. Oligosaccharides are most commonly used prebiotics in neonates that are abundantly provided through mother's milk. Commercially available products add FOS and GOS [22]. GI motility, gastric emptying is improved with prebiotic administration, this is mediated by metabolites as SCFAs. Breast fed infants and infants fed with prebiotic rich alternative feeds had decreased stool pH and increased stool SCFA. Postbiotics are bacterial products or metabolites that avoid the risk of administering live bacteria in immature intestinal barriers [17]. The most common SCFA produced by commensal includebutyratewhichservesasamajorenergysourceforcolon,roleinintestinalgrowthand development, suppressing inflammation and regulate apoptosis and encourage cellular regeneration.Earlymicrobialexposureandcolonizationinapretermguttendtopersistlonger than organism introduced at later time[3].Various subspecies of *Bifidobacterium* (*B.breve*, *B.infantis*, *B.lactis*, *B.longum*), *Lactobacillus* (*L. acidophillus, L.reutrii, L.rhamnosus, L.plantarum*) and *Streptococcus* were provenbeneficial.Varioustrialshavedemonstratedthatprobioticsininfantsandchildrenhave reduced inflammatory bowel disease IBD and prevention of antibiotics associated diarrhea. Probiotics in preterm infants have also shown to reduce NEC risk. Choice of probiotic is an important factor in preventing various gut related diseases. Most commonly used probiotics *Lactobacillus rhamnosus GG*, *L.reuterii*, *L.bulgaricus*, *L.casei*, *L.salivarius*, *Bifidobacterium infantis*and *Bifidobacterium breve* were best in reducing the incidence of diseases as NEC, diarrhea and other gut related issues in neonates. *Lactobacillus reuterii*were proposed to prevent not only the gastrointestinal tracts but also have positive effects on degree of respiratory exacerbation and infections of upper respiratory tract[37].

VII. Conclusion:

NEC primarily affects preterm infants < 32 weeks of gestation and risk increases inversely with gestational age. It remains as an important cause of death in extreme preterm neonates from 2 weeks to 2 months of age. NEC don't happen in utero and there are many modifiable risk factors such as alternative feeds, prolonged empiric antibiotics exposure, acid suppressive medications. Placenta harbors a unique microbiome in healthy pregnant women [30].Polymerasechainreaction(PCR)studiesofumbilicalcordbloodandmeconiumofnormal healthy infants revealed that even placental barrier contains microbes including *Bifidobacterium* and *Lactobacillus spp*. Meconium analysis of the fetus is more similar to the maternal oral microbiome [3]. Microbial journey between mother and fetus originates from oral cavity, where the flora moves towards the gut. The gut microbiome reaches blood stream through the help of gut associated lymphoid tissue and they reach the placenta and mammary gland [1]. This enteric and mammary gland colonization reaches the new-born during feeding. The traditional ascending pathway from cervix to placenta is observed mainly for certain pathologic conditions as chorioamnionitis, preterm labour or early onset neonatal sepsis. Dysbiosis in the preterm gut dysregulate immunity and precipitate proinflammatory reactions by increased levels of inflammatory cytokines as IL-6. NECoccursbecause of excessivetolllikereceptorssignalingwhichpromoteapoptosis,proliferationandmigrationof enterocytes. Further various other factors as gestational age, type of feeding, earlier antibiotic

treatment,lowbirthweight,mayunmaskthedevelopmentalimmaturityorreducethecapacity to handle the newly introduced microorganism in the intestinal lumen. Earlier antibiotic treatment causes reduced anaerobic *spp*, delays *Lactobacillus spp*, *Klebsiella spp*and *Staphylococcal spp.* Early administration of probiotics may help in maintaining the beneficial bacteria to prevent enterocolitis [3].Human dietary interventions aim at providing microbiological, immunological and metabolic programming of infant health [38]. Symbiotic enhancementintheguthelpsinmaturationofintestinaldefensesandmodulateinnateimmune system and gut inflammation [6].

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