UJMR, Volume 4 Number 2, December, 2019, pp 57 - 64 *ISSN: 2616 - 0668*
https://doi.org/10.47430/ujmr.1942.010

Received: 28/05/2019 **Accepted:** 25/06/2019

Pathogenesis of Necrotising Enterocolitis Associated with Faecal Microbiome among Preterm Infants: A Review

***Bello H.[1], Abdulkadir B.[2], Abdulmalik Y.[2], Murtala S.[1], Fatima M.[2], Umar L.[3] and Musa I.[1]**
[1] Department of Microbiology Kebbi State University of Science and Technology Aliero
[2] Department of Micrbiology Umaru Musa Yaradua University Katsina
[3] Department of Biology Umaru Musa Yaradua University Katsina
*Corresponding Author: bashir.abdulkadir@umyu.edu.ng (+2348167954462)

Abstract

Necrotizing enterocolitis (NEC*) is primarily a disease process of the gastrointestinal* (GI*) tract of premature neonates that results in inflammation and bacterial invasion of the bowel wall. It is the most common gastrointestinal* (GI*) emergency in neonatal intensive care units* (NICUs), *making it one of the leading causes of long-term disability in preterm infants. Despite advances in the care of premature infants, NEC remains one of the leading causes of morbidity and mortality in this population. It occurs in 1-5% of all neonatal intensive care admissions and 5-10% of all very low birth weight (<1500 g) infants. Necrotizing enterocolitis* (NEC*) is primarily a disease of premature infants, but may also be present in 10% of term and near term babies. Preterm infants show delayed colonization by "healthy commensal" organisms, especially bifidobacteria and lactobacilli. Data suggest that low colonization of Bifidobacterium and Lactobacillus in preterm Very Low Birth Weight* (VLBW*) infants may serve as a predisposing factor in microbial infection and* NEC*. The presence of a higher proportion of Proteobacteria has an association with faecal microbiome among preterm infants. Thus, the focus of this review is to provide an in-depth summary of the current knowledge regarding its association with faecal microbiome among preterm infants. Different search engines were carefully employed in analyzing scientific articles, journals, and online published data. Preventing* NEC *is instrumental in decreasing the morbidity and mortality from this gastrointestinal emergency. Human milk (breastfeeding) has been proved to be protective against* NEC *likewise probiotic supplementation has reduced both incidence and severity of necrotising enterocolitis in preterm neonates. Also, the intervention of surgery, laparotomy andthe use of stem cells in clinical neonatology is therapeutic options with huge potential.With its multifactorial pathogenesis, disease prevention remains a challenge, although, probiotic supplementation has reduced both incidence and severity of necrotising enterocolitis in preterm neonates.*

Keywords:*Faecal Microbiome, Gastrointenstinal Necrotizing Enterocolitis, Preterm, Pathogenesis*

INTRODUCTION

Necrotizing enterocolitis (NEC) is primarily a disease process of the gastrointestinal (GI) tract of premature neonates that results in inflammation and bacterial invasion of the bowel wall. Necrotizing enterocolitis (NEC) is the most common gastrointestinal (GI) emergency in neonatal intensive care units (NICUs), making it one of the leading causes of long-term disability in preterm infants (Puja and Pierre, 2012). Despite advances in the care of premature infants, NEC remains one of the leading causes of morbidity and mortality in this population. It occurs in 1-5% of all neonatal intensive care admissions and 5-10% of all very low birth weight (<1500 g) infants (Thompson The primary risk factors for NEC are prematurity, bacterial colonization, and Bizarro, 2008). At present, NEC is thought to develop in the premature host in the setting of bacterial colonization, often after administration of non-breast milk feeds, and disease onset is thought to be due in part to a baseline increased reactivity of the premature intestinal mucosa to microbial ligands as compared with the full-term intestinal mucosa (Diego *et al.*, 2016). The excessive inflammatory process initiated in the highly immune-reactive intestine in necrotizing enterocolitis extends the effects of the disease systemically, affecting distant organs such as the brain and placing affected infants at substantially increased risk for neurodevelopmental delays (Hintz *et al.*, 2011). hypoxia/altered intestinal blood flow, and enteral feeding (Erika, 2009). Prematurity with

its associated immature gastrointestinal host and blood flow regulation results in mucosal injury. Combined with enteral feeds and bacterial colonization, inflammatory mediators are released leading to a propagated inflammatory response with both pro and anti-inflammatory influences. If counter-regulatory responses to these inflammatory events are insufficient, pathologic changes to gut mucosa occur. In the preterm infant, the balance appears to favor pro inflammatory influences resulting in NEC (Troy *et al.,* 2014). NEC presents with variable symptoms which are often non-specific signs of gastrointestinal dysfunction. These signs include abdominal distension, feeding intolerance, gastric aspirates, bilious vomiting and hematochezia; with sudden progression to pneumoperitoneum and/or systemic shock and rapid death in severe cases (Claud, 2009).The focus of this review is to provide an in-depth summary of the current knowledge regarding its association with faecal microbiome among preterm infants.

Preterm Infants

Preterm infants are very unique with regard to sudden interruption of the in utero environment and microflora colonization in the intestinal tract; many preterm infants are born via cesarean section and could not have own maternal nipple feeding. This makes preterm infants nearly having no chance to get maternal generic microflora colorization in the GI tract. Furthermore, preterm infants are mandated to develop within the Neonatal Intensive Care Unit (NICU) and therefore they acquire their GI tract microbiota within the confines of the Neonatal Intensive Care Unit (NICU) where colonization is significantly influenced by iatrogenic manipulations such as the frequent administrations of diverse drugs (broad-spectrum antibiotics, opioids, and histamine-2 receptor antagonists) or numerous instrumentations such as endotracheal tubes, feeding tubes, and suctioning tubes (Lijuan *et al.,* 2015). Also, preterm infants show delayed colonization by "healthy commensal" organisms, especially *Bifidobacteria* and *Lactobacilli*. This suggests that low colonization of *Bifidobacterium* and in preterm Very Low Birth Weight (VLBW) infants may serve as a predisposing factor in microbial infection and NEC (Jacquot *et al.,* 2011).

Pathogenesis of Necrotizing Enterocolitis

The pathophysiology of classic necrotizing enterocolitis is incompletely understood. However, epidemiologic observations strongly suggest a multifactorial cause(Phani *et al.,* 2013).The vast majority of NEC occurs in infants born preterm (Lin, 2006) although up to 10% of all cases affect late preterm and term babies (Sharma and Hudak, 2013). The preterm gastrointestinal tract is potentially more susceptible to NEC as a result of immature barrier function of the intestinal mucosa, inadequate digestion of feeds, immature intestinal circulatory regulation, and immature innate immunity (Phani *et al.,* 2013).

Intestinal Epithelial Barrier

The compromised epithelial barrier and under-developed immune system when exposed to luminal microbiota that have been shaped by formula feedings, antibiotic exposure, and Cesarean delivery can lead to intestinal inflammation and sepsis (Lu *et al.,*2014).Injury to the intestinal mucosa may depend on a variety of conditions typical of prematurity, infection, hypoxia and starvation (Tran *et al.,* 2013), likewise microcirculatory dysfunction contributes to epithelial damage (Zhang, 2013).An immature epithelial barrier causes increased intestinal permeability. This, in turn, predisposes the gut to invasion of toxins and bacteria located within the gut lumen resulting in both inflammation and injury (Melissa and Patricia, 2015).

Innate Immunity: The Role of Toll-Like Receptor

Innate immunity structured components located on the epithelial surface, which play a major role in tissue repair, are the toll-like receptors (TLRs). The components of innate immunity system, TLRs display pathogen-associated molecular patterns and danger-associated molecular patterns so that they play a crucial role in the NEC progress (Gianluca *et al.,* 2014).The expression of TRL4 in preterm babies is very high, and when the premature intestine is colonized by pathologic microflora, TLR4 signal could be over activated, leading to decreased ability to repair epithelium after injury (Ozdemir *et al.,* 2014). The final effect of this TLR4-mediated response is gut barrier failure, bacterial translocation, intestinal inflammation, and finally activation of systemic inflammatory response (Leaphart *et al.,* 2007).

Gut Microbiota

Unfortunately, only a few studies have analyzed the microbiota during the entire interval between birth and NEC development. In integrating sequence and metabolomic stool analysis in preterm neonates, demonstrated that NEC does not have a uniform microbial signature.

However, a diverse gut microbiome with a high abundance of *Bifidobacteria* may protect preterm infants from disease (Stewart *et al.*, 2016).In term infants, the breast fed infant's microbiome is rich in *Bifidobacteria* and *Bacteriodes* while Preterm infants' microbiome is largely characterized by high numbers of Clostridiaceae and Enterobacteriaceae with low number of *Bifidobacteria* and *Bacteroidetes* (Vongbhavit and Underwood 2016; Arboleya*et al.*,2012).A specific pathogen has still not been identified; rather, the microbial community structure in NEC patients is distinct based on a significant decrease in diversity of microbial species with an increase in *Proteobacteria* dominance compared to other preterm infants, and a specific bloom of a single genus of *Proteobacteria* to 50% of the overall bacterial composition. While bacteria are important in the pathogenesis of NEC, NEC does not appear to be an infection in classic sense (Wang, 2009).

NEC was associated with a distinct microbiome, which was characterized by low diversity, higher abundances of *Staphylococcus* and *Clostridium*, and lower abundances of *Actinomyces* and *Corynebacterium*. La Rosa *et al*. (2014) found that Bacilli, *Gammaproteobacteria*, and *Clostridia* represented 91.7% of all bacterial sequences in the collected stool samples of neonates. Many pathogens may stimulate a picture of NEC in neonates including *Escherichia coli* , Rotavirus ,*Candida albicans, Pseudomonas aeruginosa* , Adenovirus, *Candida glabrata*, *Klebsiella,* Norovirus , *Aspergillus fumigatus ,Cronobacter sakazakii,* Astrovirus, *Shigella boydii,* Echovirus, Coagulase-negative, *Staphylococcus ,Cytomegalovirus, Clostridium* spp., Coxsackie virus, *Campylobacter,* Torovirus, *Enterobacter cloacae,* Coronavirus, *Salmonella* (Terrin *et al.*, 2014).All these data suggest that NEC may not result from a single causative species, but more likely from a currently undefined dysbiosis, that may favor TLR4 activation and pathogens translocation across the epithelium (Terrin *et al.*, 2014).

Association of NEC and Faecal Microbiome in Preterm Infants

Current evidence suggests that dysbiosis of the gut microbiota precedes the development of NEC in preterm infants (Baron *et al.*, 2017). After birth, the neonate's gut bacterial population rapidly expands, especially during the first week of life. The first bacteria that colonize normal infants are mainly aerobes or facultative anaerobes such as *Enterococcus* spp., *Streptococcus* spp. and *Staphylococcus* spp., Enterobacteriaceae, and Lactobacilli (Palmer *et al.*, 2007).Although various microbes have been cultured from blood and stools in outbreaks of NEC at single institutions, no single organism has consistently been implicated across centers. The human microbiome project (Turnbaugh *et al.*, 2007), in conjunction with technologic advances that allow for the molecular identification of a vast array of microbes that are difficult or impossible to culture from the intestine, has given new tools for generating evidence supporting the "abnormal colonization hypothesis"(Frank and Pace, 2008). Studies have also reported an absence of "pathogenic" bacteria in tissue resections from NEC infants and no difference in the total bacterial load (Abdulkadir *et al.*, 2016).Also, a study conducted by Torrazza *et al*. (2014) did confirm the presence of a higher proportion of *Proteobacteria* that the same group reported only after the diagnosis of NEC. Furthermore, with was observed that the proportion of *Proteobacteria* increased in samples collected from NEC infants over the week prior to the diagnosis of NEC. In the samples collected from the NEC infants one week before diagnosis, the proportion of *Proteobacteria* was actually lower than that observed in the matched control samples. Hence, our data raise the possibility that preterm infants not sufficiently colonized with *Proteobacteria* during the first weeks of life during which early immune protection or tolerance might develop, may not be able to modulate an adaptive immunological response to a subsequent bloom of *Proteobacteria*, and instead develop intestinal pathology consistent with NEC. Barron *et al*. (2017) in a study found no evidence that the gut microbiome, prior to the onset of disease, differentiates the clinical course of NEC. There were no differences in the pre-NEC gut microbial community between infants treated medically vs. surgically, or those with NEC totals. Furthermore, neither treatment of NEC significantly changed the gut microbiome post-NEC among the survivors. These data suggest that factors other than the gut microbiome may dictate disease severity. Abdulkadir *et al*. (2016) concluded that total bacterial load in stool samples from patients with NEC matched to healthy controls. This was not associated with the development of NEC and thus our results suggest that measuring stool bacterial load may not be a proxy measure for tissue bacterial load.

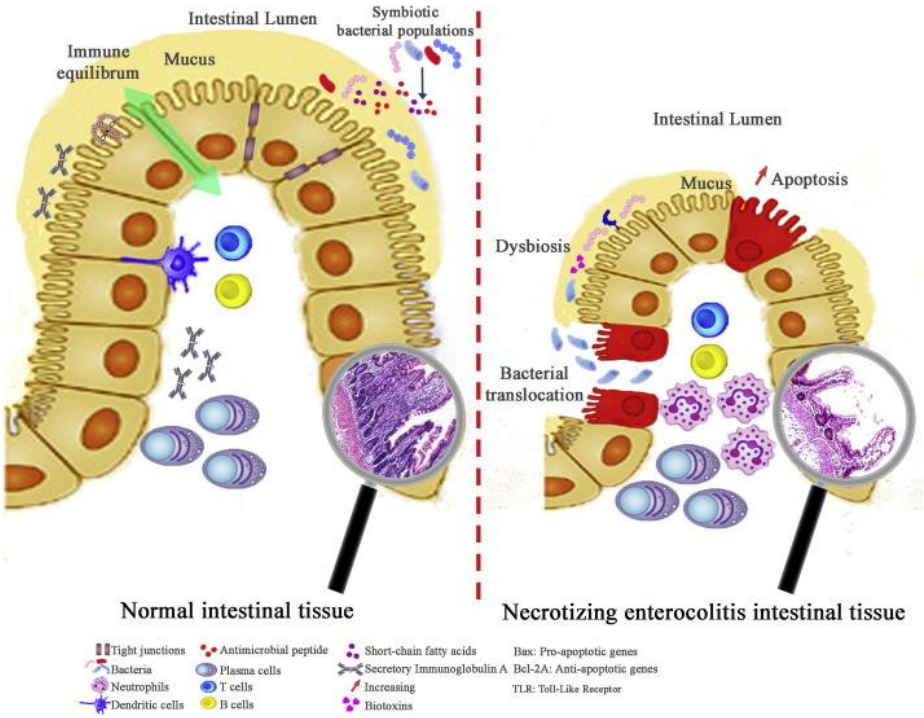

Figure 1. Diagram of normal intestinal tissue and necrotizing enterocolitis intestinal tissue. Source: Michel *et al.* (2017)

Management of NEC in Preterm Infants

There is no definitive treatment for established NEC, so, therapy is directed at giving supportive care and preventing further injury with cessation of feeding, nasogastric decompression and administration of intravenous fluids. Careful attention to respiratory status, coagulation profile, and acid-base and electrolyte balances are important (Meha *et al.,* 2016). Treatment consists primarily of supportive care including providing bowel rest by stopping enteral feeds, gastric decompression with intermittent suction, fluid repletion to correct electrolyte abnormalities and third-space losses, support for blood pressure, parenteral nutrition, and prompt antibiotic therapy (Scott, 2015). Primary prevention of NEC will be difficult till prevention of preterm birth becomes a reality (Meha *et al.,* 2016). The poorly understood pathogenesis of NEC makes it difficult to develop a cure for the illness (Torrazza *et al.,* 2014). So with improved supportive intensive care, including use of new pharmacological agents, ventilatory management, anaesthetic techniques, surgical interventions and total parenteral nutrition, the survival of infants with necrotizing enterocolitis (NEC) has steadily improved since the late 20th century(Cloherty *et al.,* 2012).

Preventing NEC is instrumental in decreasing the morbidity and mortality from this gastrointestinal emergency. Evidence based studies have shown a decrease in NEC in breast fed babies and conservative trophic feedings. However, tentatively, prevention includes the use of breast milk and probiotics (Rich and Dolgin, 2017). A 2012 policy by the American Academy of Pediatrics recommended feeding preterm infants human milk, finding "significant short- and long-term beneficial effects," including reducing the rate of NEC by a factor of two or more (Rich and Dolgin, 2017). Small amounts of oral feeds of human milk starting as soon as possible, while the infant is being primarily fed intravenously, primes the immature gut to mature and become ready to receive greater intake by mouth (Ziegler and Carlson, 2009). Human milk from a milk bank or donor can be used if mother's milk is unavailable. The gut mucosal cells do not get enough nourishment from arterial blood supply to stay healthy, especially in very premature infants, where the blood supply is limited due to immature development of the capillaries, so nutrients from the lumen of the gut are needed (Morgan *et al.,* 2014) .

Human milk has been shown to be protective against NEC. There have been a 10–20 fold reduced incidence of NEC in human milk fed preterm infants compared to formula fed infants. Lactoferrin is a multifunctional protein found in breast milk and is known to have antimicrobial and anti-inflammatory actions in the human intestine, promoting growth and proliferation of intestinal epithelium and helping in iron absorption (Sherman, 2013).

Recent studies suggest a dose-related association between human milk feeding and decreased incidence of NEC (Meinzen-Derr *et al.,* 2009). Many of the same factors present in amniotic fluid are present in human breast milk, and vary in amount with changing gestational age of the infant. Human milk may provide necessary factors for the preterm infant in the extra-uterine environment in the same way that amniotic fluid provides for the fetus in the intra-uterine environment (Sullivan *et al.,* 2010).

Probiotics are living microorganisms in food and dietary supplements which have beneficial health effects beyond their inherent nutritive value. Probiotics are most commonly *Lactobacillus* or *Bifidobacterium* strains, which are the dominant species in the intestinal microbiota of breast fed infants. Since bacteria are necessary for maturation of the intestine and appropriate containment of inflammatory responses. *Bifidobacteria* are the dominant strains in infancy, and the combination of *Lactobacilli* and *Bifidobacterium* is known to promote the growth of indigenous lactic-acid bacteria (bifidogenic effect) by formation of short- chain fatty acids as a product of the fermentation process (Abdulkadir *et al.,*2018).Thus for a premature infant it has been suggested that optimizing the bacterial flora can enhance intestinal maturation and decrease the incidence of NEC (Gionchetti *et al.,* 2000). Probiotics have been shown to be beneficial in treating inflammatory bowel diseases, rotavirus and antibiotic associated diarrhea, and *C. difficile* colitis (Gionchetti *et al.,* 2000).

Randomized controlled trials done by Lin *et al.* (2006) either with single or multicenter center was the first to prove that administration of probiotics that contain *Lactobacillus* and *Bifidobacterium* in VLBW infants reduces the incidence and severity of NEC. A meta-analysis of probiotics for preventing necrotizing enterocolitis in preterm neonates was done by Yang *et al.* (2014). This meta-analysis has shown that, regardless of gestational age and NEC stage, probiotic supplementation could significantly reduce the risk of NEC in preterm infants. Analysis also indicated that such supplementation did not increase the incidence risk of sepsis or of mortality. A study by Meha *et al.* (2016) found that probiotic supplementation has reduced both incidence and severity of necrotising enterocolitis in preterm neonates. The limitations of their study is that the choice of probiotic mixture, the dose and the frequency of dosing need to be discussed because each probiotic organism has variable rate of colonization

Up to 40% of cases of NEC require surgical intervention (Lin and Stoll, 2006) but the decision of when to intervene surgically in NEC remains unclear. Recognized indications for surgery include: pneumoperitoneum due to intestinal perforation; evidence of necrotic bowel; palpable abdominal mass; development of intestinal structure; and when there is no improvement or deterioration (including persistent hematological and biochemical derangements such as progressive thrombocytopenia and worsening metabolic acidosis) despite maximal conservative therapy (Kastenberg and Sylvester, 2013; Tepas *et al.,*2010).

Primary peritoneal drainage can be used as an alternative to laparotomy in unstable preterm infants with NEC. Various investigators have explored the benefits of primary peritoneal drainage over laparotomy. However, there is some suggestion that infants who undergo laparotomy instead of primary peritoneal drainage have a better neurodevelopmental outcome at 18 months of age (Blakely *et al.,* 2006).

Genetic markers, such as a specific mannose binding lectin genotype and variations in the toll-like receptor pathway genes, may offer a way of predicting preterm infants who are most susceptible to developing NEC (Ng and Lam 2012), because it has long been suspected that a genetic predisposition to NEC might exist, and are focusing on various biomarkers for both predicting infants who are most susceptible to NEC and early identification of NEC (Ng *et al.,* 2013). It is proposed that identification of fatty acid binding protein in the urine of preterm infants during the first few days of life can help in predicting the occurrence of NEC (Phani *et al.,* 2013).

The use of stem cells in clinical neonatology is one of the therapeutic options with huge potential, and needs further trials to establish its safety and efficacy. Amniotic fluid stem cells injected intraperitoneally resulted in improved survival, decreased the incidence and improved the macroscopic appearance and function of the gastrointestinal tract. Exciting progress in preclinical and clinical studies has brought human stem cell therapy for newborn infant one step closer to clinical translation. However, the resolution of several issues, such as safety, up-scaling of manufacturing processes, standardization methods, clinical indications, timing, dosage, and a better understanding of protective mechanisms, are required to permit safe clinical translation of stem cell therapy for newborn infants in the near future (Ahn *et al.,* 2017).

CONCLUSION

NEC continues to cause significant morbidity and mortality in large numbers of very vulnerable preterm infants. With its multifactorial pathogenesis, disease prevention remains a challenge, although, probiotic supplementation has reduced both incidence and severity of necrotising enterocolitis in preterm neonates. Yet the risk factors associated with NEC is important for the public awareness.

REFERENCES

Abdulkadir, B., Aisha, A., Hayatudeen, M.R., Musa S. K., Abdullahi, S., Sani, A., Baha'uddeen, S. D.(2018). The Impact of Probiotics as Dietary Supplementation In The

Abdulkadir, B., Nelson, A., Skeath, T., Marrs, E.C.L., Perry, J.D., Cummings, S.P. (2016). Stool bacterial load in preterm infants with necrotising enterocolitis. *Early Hum Dev*.**95**:1–2.

Ahn, S.Y.,Chang, Y.S., Sung, D.K., Sung, S.I., Ahn,J.Y., Park,W.S (2017). Pivotal Role of Brain-Derived Neutrophic Factor Secreted by Mesenchymal Stem Cells in Severe Intraventrucular Hemorrhage in Newborn Rats. *Cell Transplant*. 26(1):145-156.

Arboleya, S, Ang, L.,, Margolles, A., Yiyuan, L., Dongya, Z., Liang, X., Solís, G., Fernández, N., de Los Reyes-Gavilán, C.G., Gueimonde, M. (2012).Deep 16S rRNA metagenomics and quantitative PCR analyses of the premature infant fecal microbiota. *Anaerobe Journal*.18:378–80.

Barron, L.K., Warner, B.B., Tarr, P.I., Shannon, W.D., Deych, E., Warner, B.W. (2017). Independence of gut bacterial content and neonatal necrotizing enterocolitis severity. *J Pediatr Surg. Jun*; **52**(6):993-998. .

Blakely, M.L., Tyson, J.E., Lally, K.P. (2006). Laparotomy versus peritoneal drainage for necrotizing enterocolitis or isolated intestinal perforation in extremely low birth weight infants: outcomes through 18 months adjusted age. *Pediatrics Journal*. **117(**4):e680–e687.

Claud, E.C, Savidge, T., Walker, W.A. (2003). Modulation of human intestinal epithelial cell IL-8 secretion by human milk factors. *Pediatr Res*.**53**(3):419–425.

Cloherty, J.P., Eichenwald, E.C., Stark, A.R. (2012). Manual of neonatal care. 7th edition. Philadelphia: *Lippincott Williams and Wilkins*; 340-349.

Diego, F.N., Chhinder, P. S., David, J.H. (2016). Necrotizing enterocolitis: new insights into pathogenesis and mechanisms. *Nature Reviews Gastroenterology & HepatologyJournal***13**, 590–600.

Erika, C.C (2009). Necrotizing Enterocolitis. In: Walker G, Kleinman S, Shneider S, editors. Pediatric Gastrointestinal Disease Pathophysiology, Diagnosis, Management. 4. BC Decker;Cananda.

Frank, D.N., Pace, N.R. (2008). Gastrointestinal microbiology enters the metagenomics era. *CurrOpinGastroenterol***24**: 4–10.

Gionchetti, P., Rizzello, F., Venturi, A., Campieri, M. (2000). Probiotics in infective diarrhoea and inflammatory bowel diseases. *J GastroenterolHepatol*.; 15**(5)**:489–493.

Hintz, S.R., Kendrick, D.E, Stoll, B.J. (2005). Neurodevelopmental and growth outcomes of extremely low birth weight infants after necrotizing enterocolitis; *J Pediatr***P115**:696-703

Jacquot, A., Neveu, D., Aujoulat, F. (2011). Dynamics and clinical evolution of bacterial gut microflora in extremely premature patients. *J Pediatr;***158**(3):390–396.

Kastenberg, Z.J. and Sylvester, K.G. (2013). The surgical management of necrotizing enterocolitis. *ClinPerinatol*.**40**(1):135-148.

La Rosa, P.S., Warner, B.B., Zhou, Y., Weinstock, G.M., Sodergren, E., Hall-Moore, C.M., Stevens, H.J., Bennett, W.E., Shaikh, N., Linneman, L.A., Hoffmann ,J.A., Hamvas, A., Deych, E.,Shands, B.A., Shannon,W.D., Tarr, P.I.(2014). Patterned progression of bacterial populations in the premature infant gut. *Proc Natl AcadSci* U S A. 111:12522-7.

Leaphart, C. L., Cavallo, J., Gribar, S. C. (2007). A critical role for TLR4 in the pathogenesis of necrotizing enterocolitis by modulating intestinal injury and repair. *Journal of Immunology*, **179**:7 4808–4820

Lijuan, W. and Hung-Chih, L. (2015). Role of Intestinal Microflora on Necrotizing Enterocolitis in Preterm Infants. *J PediatrBiochem*; **5**:55–59.

Lin, J. (2004). Too much short chain fatty acids cause neonatal necrotizing enterocolitis. *Med Hypotheses*. **62**(2):291–293.

Lin, P.W., Stoll, B.J (2006). Necrotizing enterocolitis. *Lancet Journal*. **368**(9543):1271–1283.

Lu, P., Sodhi, C.P., Jia, H., Shaffiey, S., Good, M., Branca, M.F., Hackam, D.J. (2014) Animal models of gastrointestinal and liver diseases. Animal models of necrotizing enterocolitis:pathophysiology, translational relevance, and challenges. *Am J Physiol Gastrointest Liver Physiol.*;**306**:G917–G928.

Meha, T., Jaiswal, A.K., Raja, A., Sinha, A.K. (2016). Role of oral probiotics in prevention of necrotising enterocolitis in preterm neonates in a tertiary care centre in Northern India. *Int J Contemp Pediatr*. **3**:1293-6.

Meinzen-Derr, J., Poindexter, B., Wrage, L., Morrow, A.L., Stoll, B., Donovan, E.F., (2009). Role of human milk in extremely low birth weight infants' risk of necrotizing enterocolitis or death.*J Perinatol*. ;**29**(1):57–62.

Melissa, D.H. and Patricia, W.D. (2015). The role of intestinal epithelial barrier function in the development of NEC. *Tissue Barriers*. 3:1-2, e1000707.

Ng, P.C., Lam, H.S. (2012). Biomarkers in neonatology: the next generation of test. *Neonatology Journal*. **102**(2):145–151.

Ozdemir, R., Yurttutan, S., Sari. F.N. (2012). Antioxidant effectsof N-acetylcysteine in a neonatal rat model of necrotizing enterocolitis. *Journal of Pediatric Surgery* **47**:1652–1657.

Palmer, C., Bik, E.M., DiGiulio, D.B., D.A. Relman, Brown, P.O. (2007). Development of the human infant intestinal microbiota*PLoSBiol*,**5** (7) pp. 1556-1573.

Puja, S. and Pierre, F. (2012). Necrotising enterocolitis. Continuing Education in anesthesia, Critical Care and Pain. **12** (1).

Phani, K.Y., Shree, V.R., Andrew, K.E. (2013).Necrotizing Enterocolitis: Current Perspectives. *Journal of Research and Reports in Neonatology*, **4**:31-42.

Rich, B.S and Dolgin, S.E (2017). Necrotizing Enterocolitis. Pediatrics in Review. **38** (12): 552–559.

Scott, M.T., Taylor, F., Berryhill, James L. Ellenburg, Tamas J.G.,Dava S.C., Robin G., Colin, A.M. (2015).Pathogenesis of Necrotizing Enterocolitis; Modeling the Innate Immune Response. *Am J Pathol* . **185** (1).

Sharma, R., Hudak, M.L. (2013). A clinical perspective of necrotizing enterocolitis: past, present, and future. *ClinPerinatol*.**40**(1):27–51.

Sherman, M.P. (2013). Lactoferrin and Necrotizing Enterocolitis. ClinPerinatol. **40**(1):79–91.

Stewart,C.J.,Embleton,N.D.,Marrs,E.C.L.,Smith,D. P.,Nelson,A.,Abdulkadir,B.,Skeath,T.,Petros ino,J.F., Perry, J.D., Berrington, J.E., Cummings, S.P.(2016). Temporal bacterial and metabolic development of the preterm gut reveals specific signatures in health and diseases. *BioMed Central Microbiome*.4:67.

Sullivan, S., Schanler, R.J., Kim, J.H. (2010). An exclusively human milk-based diet is associated with a lower rate of necrotizing enterocolitis than a diet of human milk and bovine milk-based products. J Pediatr**156**(4):562.e1-567.

Tepas, J.J., Sharma, R., Leaphart, C.L. (2010). Timing of surgical intervention in necrotizing enterocolitis can be determined by trajectory of metabolic derangement. *J Pediatr Surg*.**45**(2):310–313.

Terrin, G., Scipione, A., De Curtis, M. (2014). Update in pathogenesis and prospective in treatment of necrotizing enterocolitis. *BioMed research international*.2014:543765.

Thompson, A.J and Bizarro, M.J (2008). Necrotizing enterocolitis in newborns: pathogenesis, prevention and management. US National Library of Medicine National Institutes of HealthPubmed. **68**(9):1227-38.

Torrazza, R.M., Li, N., Neu, J.(2014). Decoding the enigma of necrotizing enterocolitis in premature infants. *Pathophysiology***21**:21-27.

Tran, L., Ferris, M., Norris, J. (2013). Necrotizing enterocolitis and cytomegalovirus infection in a premature infant. *Pediatrics Journal*, vol. 131, no. 1, pp. e318–e322, 2013.

Troy, A.M., Holly, E., Brenda, B.P. (2014). Predicting disease severity of necrotizing enterocolitis: *How to identify infants for future novel therapies*. **(3)** 1 pp 1.

Turnbaugh, P.J, Ley, R.E., Hamady, M., Fraser-Liggett, C.M., Knight, R. (2007). The human microbiome project. *Nature **449***: 804–810.

Vongbhavit, K. and Underwood, M.A.(2016). Prevention of necrotizing enterocolitis through manipulation of the intestinal microbiota of the premature infant. *ClinTher*. 38:716–32.

Wang, Y., Hoenig, J.D., Malin, K.J, Qamar, S., Petrof, E.O., Su, J, Antonopoulos, D.A, Chang, E.B., Claud, E.C. (2009). 16S rRNA gene-based analysis of fecal microbiota from preterm infants with and without necrotizing enterocolitis. *ISME J*.**3**(8):944-954.

Yang, Y., Guo, Y., Kan, Q., Zhou, X.G., Zhou, X.Y., Li, Y. (2014). A meta-analysis of probiotics for preventing necrotizing enterocolitis in preterm neonates. *Braz J Med Biol Res*.**47**(9):804-10.

Zhang, H.Y., Wang, F., Feng, J.X.(2013). Intestinal microcirculatory dysfunction and neonatal necrotizing enterocolitis. *Chinese Medical Journal*.**126**(9) 1771–1778.

Ziegler, E.E. and Carlson, S.J. (2009). Early nutrition of very low birth weight infants. J. *Matern Fetal Neonatal. Med*.**22** (3): 191–7.