Necrotizing Enterocolitis (NEC) is the most common syndrome among preterm neonates and Very Low Birth Weight (VLBW) infants, being the cause for morbidity and mortality in many infants. However initially this was an unknown disease and was recognized as a clinical syndrome in 1960s-1970s when the mortality due to this was about 70%. At that period it was described as Idiopathic Gastrointestinal Perforation and later as NEC. This NEC is the target of study of many research works which prove that to be a multifactorial disease including factors like feeding patterns, Patent Ductus Arteriosus (PDA), reduced l-alanine secretion etc. Till date there prevails several controversies regarding this disease. I believe that it will be useful to review the research works published on NEC in order to summarize the causes and the drugs used for treating this disease in order to provide a basis for understanding and encouraging the next development in the future coming researches.

Keywords: Necrotizing Enterocolitis, Matrix Metalloproteinases, Mast cells, Necrosis, Apoptosis, Probiotics.

INTRODUCTION

Necrotising Enterocolitis (NEC) has become a major cause of mortality among preterm infants. Though some hypothesize this to be because of the bacterial colonization in the immature gut, there is yet no single proper reason suggested for NEC. It is suspected to be a complex syndrome attributed by various reasons like enteral feeding, ischemia-hypoxia, use of antibiotics, increment of mast cells, altered antibody response to antigens etc. Treatment of Enterocolitis also remains to be crucial as the reasons and diagnosis is complicated. However, following Standard Feeding Regimen (SFR), treatment with antibiotics like Cefotaxime, Ampicillin, Gentamycin, Vancomycin, Clindamycin, Metronidazole, Pentoxifylline, probiotics like Lactobacillus acidophilus and Bifidobacterium infantis etc have reduced the risk of NEC to a certain extent. This article reviews the various research works that have been carried out to understand the various causes & treatments of NEC in infants.

A research conducted by Philip J. Mc Donald, Goldham and Powell studied the altered antibody response to the ingested Antigen in the case of food protein induced Enterocolitis. They conducted this study by correlating the levels of class specific serum antibodies to the food proteins like ovalbumin, soy and cow milk as many infants have adverse reactions to cow milk or soy protein 1, 2. In their study conducted with eighteen infants the levels of Ig G, Ig M & Ig A were determined in response to the food proteins ingested. In the antibody determination carried by the modification of ELISA 3, the results showed that the IgA antibody was higher in positive challenge infants. In case of antibody Ig G, the results between positive challenge and controls were reported to be distinguishable only in the case of egg ovalbumin. Ig M was found to be decreased in positive challenge infants in response to soy protein. 4. Evidence showed that the sensation of lymphoid tissue of GIT leads to stimulation of systemic 4 and local antibody or may cause tolerance to parenterally administered antigen 5, 6 and is this sensitization was predominant in IgA 7.

The occurrence of NEC was suspected to be dependant on the patterns of Bacterial and Intestinal colonization. This was studied in CaCO-2 tissue culture and weaning rabbit models 8. This study was conducted by Simil Gupta, Glenn Morris and others due to the recognition that the environment in which a micro-organism is placed affects its virulence 9. Seventeen infants were used in the study who were diagnosed with medical or surgical NEC. In seven instances, the organism isolated from blood was also found to be present in stool and in five instances, Staphylococcus epidermidis was the blood isolate. However no clear association was established by them between the adherence grade and severity of NEC. On adhering the isolates from patients and controls using Caco-2 cell assay 10, they were unable to distinguish the adherence between them. However in weaning rabbit, NEC like injury was found to be produced by only highly adherent strains. After co-infection they reported that control gram negative isolates lost their adhering ability in Caco-2 whereas those of NEC patients remained unaffected and lost their adherence only when co infected with an enteroococcal strain from a control infant. NEC is a complex disease featured by interaction of factors like gut maturity, prior ischemic injury, inflammatory mediators and other unidentified factors and this research added one more factor called bacterial adherence to it 11.

NEC principally occurs when oral feedings are introduced to premature infants 12. A study in 1999 by Nanda Nanthakumar, Pusuyan and Walker suspected that the Necrotizing Enterocolitis was contributed by the inflammation in developing human intestine. As NEC occurs due to the immature intestinal epithelial immunologic response to bacterial stimuli 13, and often occurs in premature because of gram negative colonization; the study involved the response of immature human enterocytes to inflammatory stimuli using cytokine IL-8 as the effector response. In this research, small intestinal mucosal biopsies from infants and older children were tested and the tested specimens were assayed for succrose enzymatic activity before and after treatment with endotoxin and IL-1β. According to their report, Breast fed infants had lactobacilli and bifidobacteria as their intestinal flora whereas formula fed ones had enterobacteria and gram negative organisms 14. In this study it was proved that the IL-8 response by fetal enterocytes was greater that the response of older children when stimulated with endotoxin and IL-1β. This enhanced response explained the pathophysiology of NEC partially. This was because that the aged enterocyte adapts to the extra uterine environment and hence had decreased response to IL-1β stimulus 15.

Michael S. Caplan, Tanya Russel, Susan Kaup and Tamas sitting in 2001 attempted to study the effect of Poly Unsaturated Fatty Acid supplementation (PUFA) on NEC. Intestinal necrosis occurring in high risk population may be attributed to ischemia hypoxia, feeding and colonization. NEC was also found to have association with the phospholipid inflammatory mediator PAF 16, 17. Polysaturated fatty acids was found to be responsible for reduction of NEC in premature infants by influencing prostaglandin metabolism 19 and cytokine activity. This was done by them in neonatal Sprague Dawley rats which were delivered on 31st day of gestation and the apoptosis was...
studied by fluorescence TUNEL staining and their study concluded that
1. PUFA Supplementation reduces the incidence of NEC in rats.
2. PUFA has no effect on intestinal epithelial apoptosis. 

Thus PUFA benefits the intestinal health by reducing bacterial translocation and reducing mucosal PAF synthesis 34.

Again in 2003, a study on NEC was carried out by investigating the effect of Matrix Metalloproteinases on NEC. Mechanistic studies had shown that local immune activation leads to mucosal degradation and this is mediated by MMPs35 and these MMPs are naturally inhibited by the Tissue Inhibitor of Metalloproteinases (TIMP) which are produced by the same cell types that produce MMPs 36. In inflammatory Bowel Disease (IBD) they observed no change in production between patients and controls whereas in NEC, the TIMP and protein production was discovered to be higher in patients than controls indicating that the damaged tissues try to repair itself. Also n-Butyrate, derived from the unabsorbed carbohydrate was found to increase risk of NEC 37 and hence it was proved that the impairment of immune response in premature infants leads to bacterial colonization and hence severe tissue inflammation. These results were published by Sylvia Lin Foong Pender, Christian Braegger and McDonald 38.

Later, NEC in rat model was studied for serum cytosolic β-glucuronidase activity. This was to test the hypothesis of Morris et al 39 that cytosolic β-glucuronidase activity was elevated in intestinal injury. The study was carried out by Reed A. Dimitt, Christopher Colby, Mary Brindle, Lawrence Moss etc. in two week old Sprague Dawley rats which were divided into two groups among which one was killed immediately (I) and another group killed after ischemia and reperfusion (I/R). Microscopic injury was reported to be greater in I/R than in control group (I) where as the serum CBG activity increased to significant level only after 90 mins in I and in I/R group it remained elevated. Thus in animals containing NEC, the increased CBG level was suggested to be due to intestinal injury and similar to adult human ischemic injury. However the adult human mechanism was somewhat complex 40.

In 2004, Marie –France De La Cochetiere, Cloatilde Des Robert and Roze carried out a research to find the role of Clostridium sp. in the bacterial colonization and NEC in premature infants. Since classical methods were not able to identify specific pathogens, this research was conducted based on the molecular techniques to know the putative relationship between microorganisms & NEC. This was carried out by collecting the stools of 117 infants. After molecular studies like DNA isolation, sequence analysis, a species related to C.perfringens was detected in NEC infants & not in any of the controls and was found that 80% of the dominant fecal bacterium was unculturable 41 and certain indicators in human feces acted as an obstacle for detection of microbes. However, there was no proof to correlate the identified Clostridium sp. and NEC development 42.

Another research work in 2004 established that Intestinal Epithelial Apoptosis initiates gross Bowel necrosis in rat model of NEC. Platelet-Activating Factor (PAF) has been found to be a key factor for NEC as there was elevated level of PAF & decreased PAF Acetylhydrolase in NEC Patients. Also addition of PAF Acetylhydrolase to the formula prevented NEC in rat model. Apart from PAF, nitric acid and lipopolysaccharides were also identified as key mediators for NEC 43. In NEC models, mucosal permeability was increased leading to bacterial translocation and collapse of mucosal integrity and this was caused by PAF 44. This was because of the loss of tight functions/cells due to epithelial apoptosis and inclusion of a pan caspase inhibitor, BAF, inhibiting apoptosis reduced the event of NEC. This study was carried out by Tamas Jilling, Michele Jackson and Jing Lu 45.

A decline in NEC was reported due to the implementation of standardised feeding regimens. This was further studied by SK Patole and N De Klerk in 2004. This was done on the implementation of the standardized Feeding Regimens (SFR) in the form of Clinical Practice Guidelines (CPG).The studies were carried out in Low Birth weight infants before and after implementation of SFR 46, 47. Analysis showed that the introduction of SFR reduced the incidence of NEC by 87%. SFR was found to lead to the minimization of enteral feeding. These studies found the intercentre differences in clinical practice with fluid balance. Patent Ductus Arteriosus (PDA) and feeding regimens as the factors of NEC in VLBW infants 48. This study also reported that the implementation of SFR was important than its specific constituents 49.

The relationship between Patent Ductus Arteriosus (PDA) and NEC in very low birth weight infants was studied by Shaull Doolberg, Ayala lusky and Brain Reichman in 2005. This was done as the presence of PDA was considered as a risk factor for NEC by a mechanism called “Diastolic Steal” to create an intestinal hypoperfusion 50. Indomethacin was used for the therapeutic closure of PDA in 80% VLBW infants and the study was carried out in 8695 VLBW infants. Also the treatment was found to be effective only before the appearance of clinical signs of NEC than when treated after appearance of clinical signs 51. It was found that the risk of NEC decreased in infants who were not diagnosed with PDA 52.

In 2005, a study was conducted to test the effect of oral probiotics to reduce the incidence and severity of NEC in VLBW infants. Lactobacillus acidophilus 49 and Bifidobacterium infantis 50 were used as probiotics to reduce the incidence of NEC though no information was clearly established about the dosage, duration, safety etc. and the study was to test the reduction in the risk of NEC on administration of probiotics such as “Inforan”. This was carried out by Hung Chih Lin, Tsung wen Len, Chang Hai Tsai & William. Their study was carried out in 367 infants and the risk of NEC was lower in the probiotics group & this reduction was suspected to include mechanisms like increased barrier to translocation of bacteria and bacterial products across mucosa, competitive exclusion of potential pathogens and enhancing enteral nutrition to inhibit growth of pathogens 53.

A Neurodevelopmental and growth outcome of extremely low birth weights was studied by Susan R.Hintz, Barbara Stoll, Rosemary Higgins and others. It was seen that the surgical NEC and medicinal NEC group infants were smaller at birth than control and had significant differences in head circumference (HC). Surgical NEC groups also received diagnosis of deafness and blindness which was absent in med NEC and no NEC groups. Thus it was proved that only Surgical NEC group was associated with growth delay. This study was concentrated to a specific group of population (ELBW) and a large sample size not considered earlier. Hence ELBW infants who had received surgical treatment of NEC were reported to possess reduced weight, length and HC when compared to med NEC and controls 54.

Chronic diarrhea was associated with increased mast cells in the gut mucosa and this was termed as Mastocytic Enterocolitis and this resulted in diarrhoea & abdominal pain. A research on Mastocytic Enterocolitis was studied by Siram Jakate. Mark deemo, Mary Tobin, Rohan john and others in 2006. 70% of 47 patients with chronic diarrhea were found to have increased mast cells and cessation of diarrhoea was reported in those who received H1 & H2 receptor antagonists. The increase in mast cells was known to produce symptoms through an increase in mast cell mediator release and signals to nervous system 55. Also the mast cell population had wide fluctuations. Hence their studies were suggested to be performed during periods of symptoms and before treatment 56.

In 2007 Rob M J Moonen, Aimee D C Paulussen, Eduardo Villamor and others studied carbamoyl phosphate synthetase polymorphisms as a risk factor for NEC. Nitricoxide (NO), formed by the conversion of L-arginine to L-citrulline, was found to perturb the blood flow to intestine and maintain the mucosal integrity 57. But arginine concentrations were found decreased in premature infants with NEC 58. This study was conducted in 17 preterm infants with NEC. Their study showed that the C allele in NO synthase and homozogosity for C allele increased the risk of NEC. Also according to their study, in premature infants, the urea cycle was not fully developed 59. Along with this the decrease in arginine due to genetic variation was reported to reduce NO production and develop NEC 60.
The article by William Odita Tarnow, Jesper Brok and Amit Trivedi in 2010 established that oral probiotics reduced mortality due to NEC. They reviewed various researches conducted to examine the efficiency of probiotics in the treatment of NEC. They reported that the use of oral probiotics had reduced all-cause mortality and NEC to half and this was found to be due to the up regulation of local and systemic immunity, increment of anti-inflammatory cytokines and impermeability of the gut to bacteria and toxin. Hence the NEC pathogens were suppressed 50. Probiotics have been used in the treatment of NEC for many years. In 2011, the combination of Bifidobacterium breve and Lactobacillus casei were given as probiotics to see whether they reduced the incidence of NEC in VLBW infants. This study was carried out by Taciana Duque Braga, Gisela Alves Pontes da Silva, Pedro Israel Cabral de Lira, and Marilia de Carvalho Lima in 119 infants. They concluded that the infants in the probiotics group achieved full enteral feeding faster than the control group. They also reported no complexity in the use of probiotics unlike previous clinical trials. The mechanism was found to be due to the increase in intestinal motility.

CONCLUSION
The above article tries to bring out an overall review of all the works on NEC carried out in order to reduce the number of deaths and children in Neonatal Intensive Care Units (NICU). There are treatments which reduced the risk of NEC by 87%. However it remains impossible to completely eradicate the disease. This is because of the fact that the antibiotics which are used for the treatment of NEC becomes a cause for this on prolonged usage. This still remains controversial in the study of NEC. Also it is highly impossible to conclude any detail with a small population sample. Hence further studies are required in which a reliably large infant population will be used. Also the use of probiotics should be encouraged and further studies on the efficiency of probiotics should be investigated.

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