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Review Article

NECROTISING ENTEROCOLITIS IN INFANTS- A REVIEW

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ABSTRACT

Necrotizing Enterocolitis (NEC), the most common syndrome among preterm neonates and Very Low Birth Weight (VLBW) infants, is the cause for the 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 Perforations 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 need for further development in the fore coming researches.

Keywords: Necrotising 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 Cefolaxine, Ampicillin, Gentamycin, Vancomycin, Clindamycin, Metronidazole, Pentoxyfelline, 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 Philp.J.Mc Donald, Goldblum 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 proteins. Evidences 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 dependent on the patterns of Bacterial and Intestinal colonization. This was studied in CaCO-2 tissue culture and weanling 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 weanling 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 enterrococcal 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, Fusuyan 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 prematures due to 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 sucrose enzymatic activity before and after treatment with endotoxin and IL-1β. According to their report, Breast fed infants had *lactobacilli* and *lifidobacteria* as their intestinal flora where as formula fed ones had enterobacteria and gram negative organisms [14]. In this study it was proved than 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, Sussan 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 [16-18]. Polyunsaturated fatty acids was found to be responsible for reduction of NEC in premature infants by influencing prostroglandin 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 [20].

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 MMPs[21] and these MMPs are naturally inhibited by the Tissue Inhibitor of Metalloproteinases (TIMPs) which are produced by the same cell types that produce MMPs[22].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 [23] and hence 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 Foon Pender, Christian Braegger and McDonald [24].

Later, NEC in rat model was studied for serum cytosolic β-gluconidase activity. This was to test the hypothesis of Morris et al [25] that cytosolic β-gluconidase activity was elevated in intestinal injury. The study was carried out by Reed A. Dimmit, 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 [26].

In 2004, Marie –France De La Cochetiere, Clotilde 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.perfringrens* was detected in NEC infants & not in any of the controls and was found that 80% of the dominant fecal flora was unculturable [27] and certain inhibitors 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 [28].

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 [29, 30].In NEC models, mucosal permeability was increased leading to bacterial translocation and collapse of mucosal integrity and this was caused by PAF[31]. 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 [32].

A decline in NEC was reported due to the implementation of standardized 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[33,34]. Analysis showed that the introduction of SFR reduced the incidence of NEC by 87%. SFR was to 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[35].This study also reported that the implementation of SFR was important than its specific constituents [36].

The relationship between Patent Ductus Arteriosus (PDA) , indomethacin and NEC in very low birth weight infants was studied by Shaul Dollberg, 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 hypo perfusion [37]. 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 [38].It was found that the risk of NEC decreased in infants who were not diagnosed with PDA[39].

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* [40] and *Bifidobacterium infantis* [41] 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 "Infloran". 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[42].

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 [43].

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 Sriram jakate. Mark demeo, 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 diarrhea 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 [44]. Also the mast cell population had wide fluctuations. Hence their studies were suggested to be performed during periods of symptoms and before treatment [45].

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. Nitric oxide (NO), formed by the conversion of L-arginine to L-citrulline , was found to pertain the blood flow to intestine and maintain the mucosal integrity [46] . But arginine concentrations were found decreased in premature infants with NEC [47]. This study was conducted in 17 preterm infants with NEC. Their study showed that there was an increase in C allele in NEC and homozygosity for C allele increased the risk of NEC. Also according to their study, in premature infants, the urea cycle was not fully developed [48]. Along with this the decrease in arginine due to genetic variation was reported to reduce NO production and develop NEC [49].

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 systematic 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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REFERENCES

1. Collins-Williams C. Incidence of milk allergy in pediatric practice. J. Pediatr. 1956; 48:39
2. Gerrard JW, MacKenzie JWA, Goluboff N, Garson JZ, Maningas CS. Cow's milk allergy: prevalence and manifestations in an unselected series of newborns. Acta.Paediatr.Scand. 1973; 234:1.
3. Ahlstedt S, Carlsson B, Hanson LA, Kaijser B, Mattsby-Baltzer I, SohlAkerlund. Class specific antibodies. Scand.J.Immunol.1978; 8 Suppl 7:119
4. Wostmann BS, Pleasants JR, Bealmear P. Dietary stimulation of immune mechanisms. Fed Proc.1971; 30:1779.
5. Hanson DG, Vaz NM, Laia LCS, Hornbrook MM, Lynch JM, Roy CA. Inhibition of specific immune responses by feeding protein antigens. Int. Arch .Allergy Appl Immun. 1977;55:526
6. Thomas HC, Parrott DMV. The induction of tolerance to soluble prote in antigen by oral administration Immunology.1974;27:631.
7. Philip J Mcdonald, Randall M, Goldblum, Greggory J Van sickle, Geraldine keating powelu. Food Protein-induced Enterocolitis: Altered Antibody Response to Ingested Antigen.1984;18:751-755.
8. Stoll BJ, Kanto Jr WP, Glass RI, Nahmias AJ, Brann Jr AW.Epidemiology of Necrotizing Enterocolitis: a case control study. J.Pediatr. 1980;96:447-451
9. Mackowiak PA, normal microbial flora. N.Engl .J .Med .1982; 307:83-93 .
10. Pinto M, Robine-Leon S, Appay MD, Kedinger M, Triadou N, DussauLx E, Lacroix B, Simon-Assmann P,Haffen K, Fogh J,Zweibaum A. Enterocyte-like differentiation and polarization of the human colon carcinoma cell line Caco-2 in culture. Biol .Cell.1983;47:323-330
11. Kliegman RM, Fanaroff AA. Necrotizing Enterocolitis. N. Engl. J. Med.1984; 310:1093-1103
12. Pinaki Panigrahi, Sunil Gupta, Ira H Gewolb, Glenn Morris JR. Occurrence of Necrotizing Enterocolitis May Be Dependent on Patterns of Bacterial Adherence and Intestinal Colonization: Studies in Caco-2 Tissue Culture and Weanling Rabbit models.1994;36:115-121
13. MacKendrick W, Caplan M. Pediatr. Clin. North Am. 1993;40:1047
14. Bakner S E, Scott P H, Wharton BA.Arch. Dis. Child. 1989; 64:1678–1674.
15. N.Nanda Nanthakumar, Robert D, Fusunyan, Ian Sanderson, Allan Walker W. Inflammation in the developing human intestine:A possible pathophysiologic contribution to necrotizing Enterocolitis. Med sci.1999;97:6043-6048
16. Caplan M, Hsueh W, Kelly A, Donovan M . Serum PAF acetylhydrolase increases during neonatal maturation. 1990;39:705–714
17. Hsueh W, Caplan MS, Sun X, Tan X, MacKendrick W, Gonzalez-Crussi F .Platelet-activating factor, tumor Necrosis factor, hypoxia and Necrotizing Enterocolitis. Acta. Paediatr. 1992;396:11–17
18. Gonzalez-Crussi F, Hsueh W . Experimental model of ischemic bowel Necrosis. The role of platelet-activating factor and endotoxin. 1986; 112:127–135
19. Carlson S.E, Montalto M.B, Ponder D.L, Werkman S.H, Korones S.B. Lower incidence of Necrotizing Enterocolitis in infants fed a preterm formula with egg phospholipids.Pediatr.Res.1998;44:491.
20. Michael S. Caplan, Tanya Russell, Yu Xiao, Michael Amer, Susan Kaup, Tamas Jilling . Effect of Polyunsaturated Fatty Acid (PUFA) Supplementation on Intestinal Inflammation and Necrotizing Enterocolitis (NEC) in a Neonatal Rat Model.2001; 49:647-652.
21. Pender S.L, Tickle S.P, Docherty A.J, Howie D, Wathen N.C, MacDonald T.T. A major role for matrix metalloproteinases in T cell injury in the gut. J.Immunol.1997; 58:1582–1590.
22. Birkedal-Hansen H, Moore W.G, Bodden M.K, Windsor L.J, Birkedal-Hansen B, DeCarlo A, Engler J.A. Matrix metalloproteinases: a review. Crit. Oral Biol Med.1993; 4:197–250
23. Kien C.L.Digestion, absorption, and fermentation of carbohydrates in the newborn. Clin. Perinatol.1996; 23:211–228.
24. Sylvia Lin Foon Pender, Christian Braegger, Ute Günther, Giovanni Monteleone, Martin Meuli, Detlef Schuppan,Thomas Thornton Macdonald. . Matrix Metalloproteinases in Necrotising Enterocolitis.J. Pedtr.2003;54:160-164
25. Morris S, Hays W, Enomoto M, Glew R, Feddersen R, Fry D, Morris D. Serum cytosolic beta-glucosidase elevation and early ischemic injury to guinea pig small intestine. 1999; 125:202–210.
26. Reed A. Dimmitt, Robert Glew, Christopher Colby, Mary Brindle, Erik Skarsgard, Lawrence Moss R. Serum Cytosolic Glucosidase Activity in a Rat Model of Necrotizing Enterocolitis.J.Pedtr.2003;54:462.
27. Suau A, Bonnet R, Sutren M, Godon J.J, Gibson G.R, Collins M.D, Doré J. Direct analysis of genes encoding 16S rRNA from complex communities reveals many novel molecular species within the human gut. Appl .Environ Microbiol 1999; 65:4799–4807.
28. Marie-France De La Cochetière, Hugues Piloquet, Clotilde Des Robert,Dominique Darmaun, Jean-Paul Galmiche, And Jean-Christophe Rozé. Early Intestinal Bacterial Colonization and Necrotizing Enterocolitis in Premature Infants: The Putative Role of Clostridium. J.Pedtr. 2004; 56: 366-370.
29. Caplan M.S, Sun X.M, Hseuh W, Hageman J.R. Role of platelet activating factor and tumor Necrosis factor in neonatal Necrotizing Enterocolitis. J. Pediatr. 1990; 116: 960 –964.
30. Caplan M.S, Kelly A, Hsueh W. Endotoxin and hypoxia-induced intestinal Necrosis in rats: the role of platelet activating factor. Pediatr. Res.1992;31:428–434.
31. Schmitz H, Fromm M, Bentzel C.J, Scholz P, Detjen K, Mankertz J, Bode H, Epple H.J, Riecken E.O, Schulzke J.D. Tumor Necrosis

factor- (TNF) regulates the epithelial barrier in the human intestinal cell line HT-29/B6.J.Cell Sci.1999;112:137–146.

32. Tamas Jilling, Jing Lu, Michele Jackson,Michael S. Caplan. Intestinal Epithelial Apoptosis Initiates Gross Bowel Necrosis in an Experimental Rat Model of Neonatal Necrotizing Enterocolitis.J.Pedtr.2004;55:622-629.
33. Bell M.J, Ternberg J.L, Feigin R.D. Neonatal Necrotizing Enterocolitis. Therapeutic decisions based upon clinical staging. Ann. Surg.1978; 187:1–7.
34. Walsh M.C, Klieg man R.M. Necrotising Enterocolitis: treatment based on staging criteria. Pediatr.Clin North Am.1986; 33:179–201.
35. Uauy R.D, Fanaroff A.A, Korones S.B. Necrotizing Enterocolitis in very low birth weight infants: biodemographic and clinical correlates. National Institute of Child Health and Human Development Neonatal Research Network. J. Pediatr .1991; 119:630–638.
36. Patole S.K, De Klerk N. Impact of standardised feeding regimens on incidence of neonatal necrotising Enterocolitis: systematic review and meta-analysis of observational studies.2005;90:147-151.
37. Knight D.B. The treatment of patent ductus arteriosus in preterm infants: a review and overview of randomized trials. Semin. Neonatol.2001; 6:63–73.
38. Clyman R.I. Recommendations for the postnatal use of indomethacin: an analysis of four separate treatment strategies. J.Pediatr. 1996;128:601–607.
39. Shaul Dollberg, Ayala Lusky, and Brian Reichman, in collaboration withthe Israel Neonatal Network. Patent Ductus Arteriosus, Indomethacin and NecrotizingEnterocolitis in Very Low Birth Weight Infants:A Population-Based Study.J.Pedtr Gastro and Nutri.2005;184-188.
40. Dani C, Biadaioli R, Bertini G, Martelli E, Rubaltelli F.F. Probiotics feeding in prevention of urinary tract infection, bacterial sepsis and Necrotizing Enterocolitis in preterm infants. A prospective double-blind study.Biol Neonate. 2002; 82:103–108.
41. Hoyos A.B. Reduced incidence of Necrotizing Enterocolitis associated with enteral administration of Lactobacillus acidophilus and Bifidobacterium infantis to neonates in an intensive care unit. Int. J .Infect Dis.1999; 3:197–202.
42. Hung-Chih Lin, Bai-Horng Su, An-Chyi Chen, Tsung-Wen Lin, Chang-Hai Tsai, Tsu-Fuh Yeh, William Oh. Oral Probiotics Reduce the Incidence and Severity of Necrotizing Enterocolitis in Very Low Birth Weight Infants.J.Pedtr.2005;115:1-4.
43. Susan R. Hintz, Douglas E. Kendrick, Barbara J. Stoll, Betty R. Vohr, Avroy A. Fanaroff, ; Edward F. Donovan, W. Kenneth Poole,Martin L. Blakely, Linda Wright, Rosemary Higgins for the NICHD Neonatal Research Network .Neurodevelopmental and Growth Outcomes of Extremely Low Birth Weight Infants After Necrotizing Enterocolitis.J.Pedtr.2005;115:696-703.
44. Wood J.D. Enteric neuroimmunophysiology and pathophysiology. Gastroenterology. 2004;127:635–657.
45. Jakate, Mark Demeo, Rohan John, Mary Tobin,Ali Keshavarzian. Mastocytic Enterocolitis: Increased Mucosal Mast Cells in Chronic Intractable Diarrhea. Arch. Pathol Lab Med.2006; 130:362-367.
46. Reber K.M, Nankervis C.A, Nowicki P.T. Newborn intestinal circulation. Physiology and pathophysiology.Clin.Perinatol.2002; 29:23–39.
47. Wu G, Jaeger L.A, Bazer F.W, Rhoads J.M. Arginine deficiency in preterm infants: biochemical mechanisms and nutritional implications. J .Nutr Biochem.2004;15:442–451.
48. Summar M.L, Hall L, Christman B, Barr F, Smith H, Kallianpur A, Brown N, Yadav M, Willis A, Eeds A, Cermak E, Summar S, Wilson A, Arvin M, Putnam A, Wills M, Cunningham G. Environmentally determined genetic expression: clinical correlates with molecular variants of carbamyl phosphate synthetase I. Mol Genet Metab 2004; 81:S12–S195.
49. Rob M.J. Moonen, Aimee D.C. Paulussen, Nicole Y.P. Souren, Alfons G.H. Kessels, M. Estela Rubio-Gozalbo, Eduardo Villamor. Carbamoyl Phosphate Synthetase Polymorphisms as a Risk Factor for Necrotizing Enterocolitis.J.Pedtr.2007;62:188-190
50. William Odita Tarnow-Mordi, Dominic Wilkinson, Amit Trivedi , Jesper Brok. Probiotics Reduce All-Cause Mortality and Necrotizing Enterocolitis: It Is Time to Change Practice.J.Pedtr.2010;125:1068-1070.