A Study of Histopathological Findings in Neonatal Necrotizing Enterocolitis in Early and Late Term Infants

O.H. Radhika Krishna[1], Mohammed Abdul Aleem[2], Mandakini Kotaiah[3], Ramesh Reddy Kota[4]

[1, 2] Department of Pathology, Osmania Medical College, Koti, Hyderabad, Telangana, India.
[3, 4] Department of Paediatric Surgery, Niloufer Institute of Paediatrics, Red Hills, Hyderabad, Telangana, India.

ABSTRACT

BACKGROUND

Neonatal necrotising enterocolitis (NNEC) is very common among premature infants. However, its incidence in term babies has also been on the rise. It is a potentially devastating condition with variable mortality. The diagnosis and management of NNEC is clinically supported by modified Bell's grading. In cases where surgical resection of bowel is performed, histological findings are rarely given much importance. In this study, we have studied the histopathology of resected bowel in NNEC in term babies and correlated these findings with the age of the neonate and also with modified Bell's grading.

METHODS

25 cases of small bowel specimens of term infants received at the pathology department of a tertiary paediatric referral hospital were studied in detail for gross and microscopic features. The histological parameters considered were transmural coagulative necrosis, granulation tissue, crypt distortion, pseudomembrane formation, villous atrophy and pneumatosis intestinalis. These findings were compared with the age of the neonates and also correlated with the modified Bell's grading. The clinical presentation and histology were also compared in early presenting term neonates less than a week in age and term infants of more than a week.

RESULTS

We found early neonates of age less than a week to have higher Bell's grading and more severe histology.

CONCLUSIONS

Term neonates also present with severe NNEC, requiring surgical resection of the bowel. Early presentation and higher Bell's grading are associated with more severe histopathological changes.

KEYWORDS

NNEC, Term Neonates, Bell's Grading, Histopathology

Corresponding Author:
Dr. O.H. Radhika Krishna,
Department of Pathology,
Osmania Medical College,
Koti, Hyderabad,
Telangana, India.
E-mail: othululuradhika@yahoo.co.in

DOI: 10.18410/jebmh/2021/35

How to Cite This Article:
Krishna OHR, Aleem MA, Kotaiah M, et al. A study of histopathological findings in neonatal necrotizing enterocolitis in early and late term infants. J Evid Based Med Healthc 2021;8(04):184-189. DOI: 10.18410/jebmh/2021/35

Submission 18-09-2020,
Peer Review 25-09-2020,
Acceptance 09-12-2020,
Published 25-01-2021.

BACKGROUND

Charles Billard gave the first description of (NNEC) neonatal necrotizing enterocolitis in 1883. He termed it as "gangrenous enterocolitis" or "malignant enteritis". He described necrosis and inflammation of the intestinal tract in a small infant. This was followed by a report of 25 patients with gangrenous enterocolitis in 1850.[1]

With the increased survival of (VLBW) very low birth weight and premature babies, the incidence of NNEC has been increasing and at present ranges from 1 – 5 % of all admissions in the neonatal intensive care unit.[2] Aetiology is uncertain and strikes in 1000 live births and the most affected are premature infants. Since 1973, there has been recognition that NEC can exist in full term infants[3] accounting for about 10 % of babies with NEC.[4] Bloody diarrhea, bilious vomiting and abdominal distension are the common clinical features of NNEC. Pneumatosis intestinalis and pneumoperitoneum are the radiological features.

NEC was called infectious enteritis in the past and ileal perforation was reported in these cases. In 1953, Schmidt and Quaiser coined the term "Newborn NEC".[5] The recent understanding of clinical and radiological features was described by Berdon of New York Babies Hospital. He described 21 cases of infants with NEC IN 1964.[6] The reasons for infants being susceptible to gut injury were described as gut immaturity, lack of barrier function or dysmotility, ischemia, inflammatory cytokines, enteric feeding, medications causing altered gut flora or associated secondary diseases. Tissue hypoxia causes further decrease in the gut motility and due to continued feeding changes occur in the gut flora. This altered gut flora causes mucosal damage. Septicemia occurs in 30 % of patients with NNEC secondary to entry of gut bacteria into systemic circulation.

Staging of NEC was given by Bell in 1978. This grading was based on its severity. This grading was modified and a better system for understanding the severity and prognosis was given by Walsh & Kliegman[7]. Histopathological findings are seldom studied in detail and influence of infants age on histology is not researched. Present study attempts to correlate the histopathological findings with modified Bell's grading[8] and also assess its utility in predicting the prognosis.

METHODS

This is retrospective study of 5 years (June 14 to June 18) at a tertiary pediatric referral hospital. 25 cases of resected bowel specimens of NNEC of full-term infants received at the Department of Pathology at a tertiary pediatric referral center were studied in detail for relevant histopathological findings. The cases were categorized into early onset NNEC (0 - 7 days) and late onset NNEC (8 - 28 days). Clinical features at the time of presentation were compared in the early and late presenting infants. Gross microscopic details were analyzed in all the cases. Six histological parameters were studied on hematoxylin and eosin stained slides and the findings tabulated. These findings were then compared in the early and late neonates and also with a higher or lower modified Bell's grading. Bell's grade Ia, Ib, IIa, IIb was considered as lower grading and IIIa / IIIb as higher grading. The histological parameters studied in detail included, transmural coagulative necrosis, granulation tissue, crypt distortion, pseudomembrane formation, villous atrophy and pneumatosis intestinalis.

RESULTS

13 cases among the 25 cases [52 %] were early neonates in the age group of 0 - 7 days and 12 cases (48 %) were late neonates in the age group of 8 - 28 days. In the early neonates' clinical findings of abdominal distension, bilious vomiting and decreased appetite were common in all the cases [100 %]. On radiology, pneumatosis intestinalis was seen in 3 cases [23 %] and pneumoperitoneum in 5 cases [38.4 %]. In the late neonates, however, abdominal distension and bilious vomiting were noted in 11 [91.6 %] cases and decreased acceptance of food in 2 [16.6 %] cases. Pneumatosis intestinalis was seen in 1 [8.3 %] case and pneumoperitoneum in 2 [16.6 %] cases on radiology. [Image 1, Image 2]

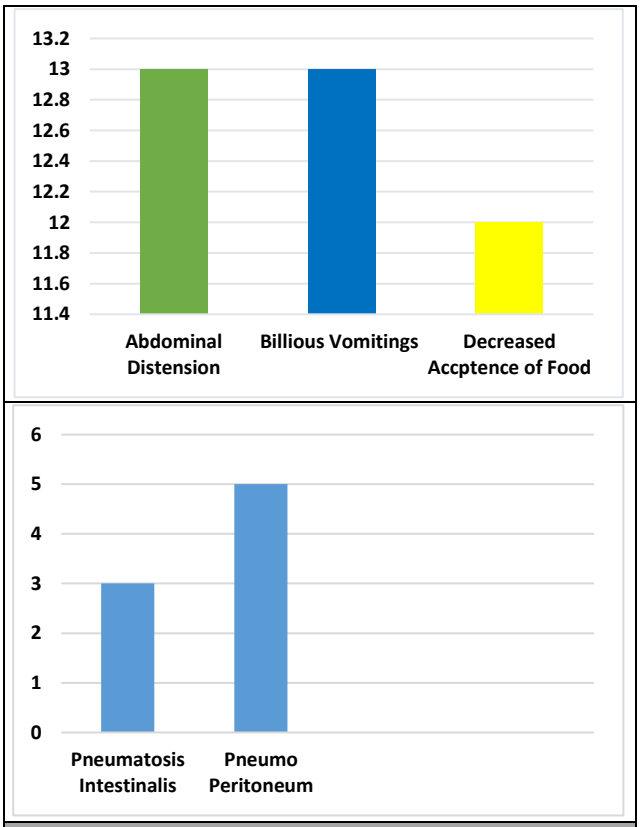
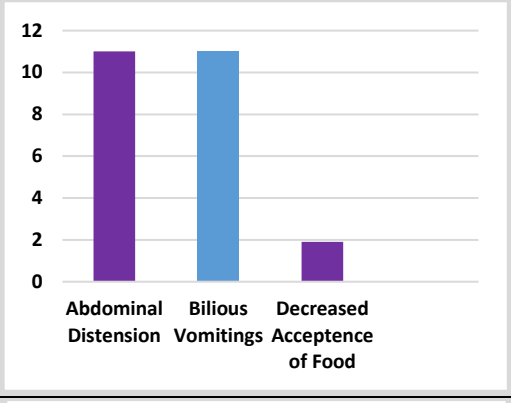


Image 1. Clinical Presentation & Imaging in Early Neonates

Histological Parameter	Early Neonates[13]	Late Neonates[12]
Transmural necrosis	10	8
Granulation tissue	8	4
Crypt distortion	8	5
Pseudomembrane formation	7	6
Villous atrophy	7	6
Pneumatosis intestinalis	0	1

Table 1. Comparison of Histological Findings of Neonatal Necrotising Enterocolitis in Early and Late Neonates



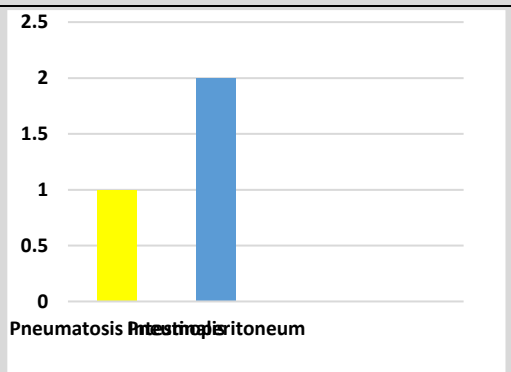
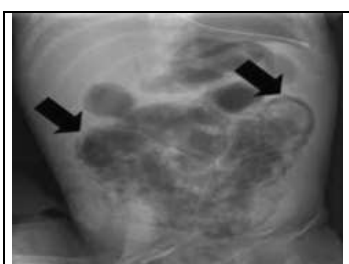
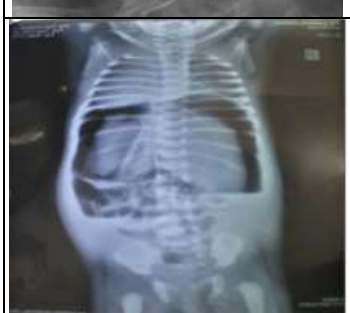


Image 2. Clinical Presentation & Imaging in Late Neonates



Pneumatosis Intestinalis

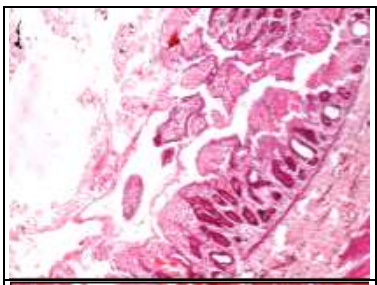


Pneumoperitoneum

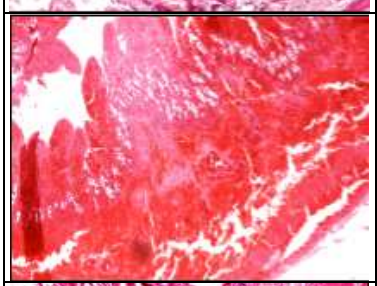
Jejunal Segment Gross

Cut Section

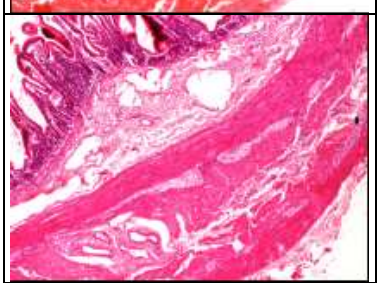
Image 3. Imaging and Gross



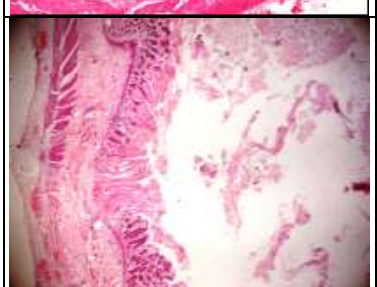
Pseudomembrane



Transmural Haemorrhagic Necrosis



Pneumatosis Intestinalis



Villous Necrosis

Image 4. Histological Findings

Histological Parameter	Higher Bell's Grading Total No. N = 9	Early Neonates Higher Bell's Grading n =7	Late Neonates Higher Bell's Grading n = 2	Lower Bell's Grading Total No. n = 16	Early Neonates Lower Bell's Grading n = 4	Late Neonates Lower Bell's Grading
Transmural Necrosis	9	7	2	16	5	10
Granulation Tissue	8	7	1	9	4	5
Crypt Distortion	8	6	2	10	5	5
Pseudomembrane Formation	7	6	3	6	2	4
Villous Atrophy	8	7	2	5	4	1
Pneumatosis intestinalis	1	0	1	0	0	0

Table 2. Bell's Grading Compared with Histological Findings of NNEC in Early and Late Neonates

On comparing Bell's grading with histology, we found the histological parameters to be associated with higher Bell's grading and therefore commoner in early neonates. [Table 2]

Advanced stage of Bell's grading IIIa / IIIb was noted in 9 cases out of 25 [36 %] among which 7 [53.8 %] were early cases and 2 [16.6 %] were late, and mild Bell's grade Ia, Ib,

lla, llb was seen in 16 cases [64 %], in which 4 [30.7 %] were early and 7 [58 %] were late neonates.

Grossly ileum and jejunum were involved. Bowel wall thickening along with vascular congestion were most common gross features. External surface showed fibrinous exudates on the serosa. Few cases showed bowel wall perforation. Mucosal changes included ulceration and cobble stone appearance.

Length of bowel ranged from 1 - 5 cm, bowel loops were congested or plum coloured haemorrhagic and oedematous. Skipped lesions were common. Mesentery appeared normal. Cut section of the bowel showed haemorrhage and with thinned out bowel wall and loss of rugosity of mucosa. All of the findings were more severe and more common in early onset cases as compared to late onset. [Image 3]

Histological parameters assessed on microscopy were transmural necrosis, presence of granulation tissue, crypt distortion, pseudomembrane formation and villous atrophy. [Image 4]. Transmural coagulative necrosis was commonest microscopic finding 10 / 13 in resected specimens of bowel in early neonates. 8 cases showed presence of granulation tissue and crypt distortion. 7 cases had pseudomembrane formation and villous atrophy. Late group showed lesser degree of histological changes. 8 cases had transmural necrosis. Villous atrophy and pseudomembrane formation in 6 cases. crypt distortion and granulation tissue in 4 cases. Pneumatosis intestinalis was seen in one case. [Table 1]

DISCUSSION

Increasing incidence of NNEC in full term infants is on the rise. Many a times, no risk factors are identified. The two well accepted reasons of NNEC are prematurity and onset of enteral feeding. Full term infants are responsible for 5 - 25 % of the cases according to literature. NNEC is most of the time managed conservatively with supportive antibiotic therapy. Infants with NEC require surgical intervention when necrosis extends through the bowel wall and results in perforation. Additional surgical indications include peritonitis, the presence of an abdominal mass, ascites, intestinal obstruction fixed dilated loop, or unremitting clinical deterioration for an extended period of time. A sudden change in vital signs, such as tachycardia, hypothermia, or impending shock might be a sign of perforation and also warrants surgery.

Mucosal permeability plays an important role in NNEC, as was noted in the animal models. Bacterial entry into the submucosa occurs secondary to an increased mucosal permeability. This will initiate a cascade of inflammatory mediators resulting in collapsed integrity of the mucosa.[9]

The bacterial colonisation of the immature gut of the infant leads to an extensive immune response and a cytokine release leads to mesenteric vasoconstriction and intestinal ischemia. The immature gut of the infants differs from mature in different ways, like reduced immune function, decreased motility, limited intestinal epithelial barrier, low digestive and absorptive capacity and altered gut flora. Intestinal ischemia is the primary cause for the gut injury. It may result from systemic hypo perfusion, reduced splanchnic perfusion resulting from systemic hypoxia or effect of inflammatory mediators on blood vessels. Unlike infectious enterocolitis no specific organism is cultured in NNEC.

Despite medical and surgical management, the mortality has been ranging between 20 – 50 %.[10] Combination of factors to initiate and propagate the disease and pathogenesis is not completely understood. It is essential that the disease is recognized early so that early management is initiated, and complications and surgery is prevented.

At our institution most cases are referred with typical clinical history of neonatal enterocolitis (NNEC), based on their gastric residue, and / or vomiting, abdominal distention and blood in their stools, together with the radiological evidence of pneumatosis intestinalis, pneumoperitoneum, portal venous gas. Finally, these babies are grouped into 3 stages as / per the modified Bell's classification. Cases are managed conservatively with fluid and infection management protocols. Progress of the babies is monitored on proforma which involves the vital parameters, complete blood picture for total neutrophil and platelet count, C reactive protein levels, blood culture, abdominal girth measurement, absence of bowel sounds palpation of lump and haematochezia. Cases are taken up for surgery based on the clinical deterioration of patient condition.

Out of 25 cases of NNEC studied over a period of 5 years, 13 cases were early onset NNEC (0 - 7 days) and 12 were late onset (8 - 28 days).

The clinical symptoms studied were abdominal pain, bilious vomiting and decreased feeding which were seen in all cases of early onset NNEC while in the late onset similar findings were seen in most cases. Imaging revealed pneumatosis intestinalis and pneumoperitoneum in half cases of early onset NNEC and only one case showed pneumatosis and two cases showed pneumoperitoneum in late onset NNEC.

Clinical features were comparable but radiological features were subtle in late onset NNEC. Out of the 25 cases, higher Bell's grade IIIb was noted in 9 cases and grade I & II in 16 cases. Ileum and jejunum were commonly involved in our study similar to the literature. Histology revealed more severe changes of transmural necrosis, granulation tissue, villous atrophy and pseudomembrane formation in cases with higher grade on Bell's classification and the lower grades showed only congestion and inflammation.

NEC is very common in premature infants. They present early. In our study, 25 cases of term infants were included. Cases presented over 18 days of birth. In 1978 Bell devised a grading system for NEC. It was subsequently modified by Walsh and Kliegman.[11] This modified Bell's grading is used in radiological stratification of patients and accordingly management is initiated. We in this study dealt primarily with resected gangrenous bowel specimens and have analyzed the microscopic features.

On gross examination ileum and jejunum were the specimens we received; no specimen of large bowel was received by us during the study period. However, any part of the bowel, small or large may be affected. In very severe

case, the entire small and large bowel may be involved called NEC totalis and has very high morbidity. Gross inspection findings were in accordance with the literature. The resected bowel in NEC is dilated, dull green to grey to black in colour when there is extensive transmural involvement of the bowel, it leads to perforation which occurs at the meeting point of necrotic and normal bowel.

The histologic appearance of the lesions is dependent on the stage and severity of disease at the time of examination. The histological hallmark of NEC is ischemic or coagulative necrosis. Coagulative necrosis is defined as loss of nuclei and diminished cytoplasmic staining but with relatively preserved, 'ghost-like' crypt-villus histoarchitecture. Similar findings were noted by Ramon et al. in their study.[12]

Arterial blood supply to the ileocecal region is away from the superior mesenteric artery. Thromboembolism of the mesenteric artery is a known risk factor associated with ischemic necrosis. large vessel occlusion is rarely seen in NNEC, though necrosis of the bowel is ischemic necrosis. Infarction is arterial in origin, single and in accordance with distribution of the arterial blood supply. Pathology in NEC is venular in origin and the process is inflammatory, also multiple random areas are involved. Tissue damage over a longer period of time and several days duration is suggested by reparative changes like, regenerating epithelium and granulation tissue formation. These changes are also associated with a higher Bell's grading.[13]

With more advanced disease, mucosal ulcerations deepen and become covered with pseudomembrane consisting of fibrin, necrotic epithelium and inflammatory cells. pseudomembrane formation is sign of more severe disease seen more commonly associated with higher Bell's grading.

Intestinal pneumatosis though rare, is a finding very characteristic of NNEC. The formation of gas bubbles within the intestinal wall, most likely resulting from the fermentation of intraluminal contents by bacteria, is associated more with NEC than with any other necrotizing conditions affecting the intestine.[14] cystic dilatation of lymphatic vessels filled with gas is not responsible for pneumatosis intestinalis. These are gas filled pseudocysts with histiocytic bordering along with foreign body giant cells, that become mesothelialised in the subserosa. Lymph vessels would thus not have significance for the morphogenesis of this disease.[15]

Reduction of pH by fermentation of lactose by P-galactosidase a bacterial product has been implicated in the pathogenesis of NEC. This ability of the colonising bacteria to cause NEC is not correlated. The endemic cases of NEC are not consistently associated with a single infectious agent or with a particularly virulent organism that produces highly damaging toxins or that displays great entero-invasive or entero-aggregative ability.[16]

The intestine appears congested, discoloured, thickened, dark grey to black in colour and gangrenous with perforation. Microscopy reveals vascular congestion, oedema and inflammation in the mucosa and submucosa, villous and crypt distortion, pseudomembrane formation and pneumatosis intestinalis was a very rare finding in our study, seen in only one case (4 %).

No histopathological grading is available for NEC. Jilling[17] using a rat model graded NEC from 0 to 4, with grade 0 representing intact morphology, grade 1 showing sloughing of the tip of villi, grade 2 with mild necrosis of villi, grade 3 with loss of villi and grade 4 representing complete destruction of the intestinal mucosa.

They used an apoptosis detection system, caspase enzyme activity detection using enzyme assay and caspase inhibitor Boc-Asp (OMe) - fluoromethyl ketone (BAF) in their study. They concluded that abundant apoptosis of the intestinal epithelium precedes widespread tissue damage in an experimental model of neonatal NNEC. Given that caspase inhibition protects neonatal rats from Formula-Feeding and Cold / Asphyxia Stress (FFCAS) induced intestinal injury, apoptosis might be the underlying cause of subsequent gross mucosal damage and better understanding of signalling and execution mechanisms that led to programmed cell death in enterocytes might provide the basis for the design of future therapeutic and or preventative strategies for NNEC. A similar study on the resected bowel specimens of NNEC of term infants is needed for further research as FFCAS plays an important role in NNEC in term infants.

CONCLUSIONS

The present study is a simple effort to understand the histopathological findings of NNEC in resected bowel specimens of full-term neonates. Higher Bell's grading is associated with more histological changes. We found a significant correlation between higher Bell's grading and increased coagulative necrosis. Histological changes were more commonly present and more severe in early onset NNEC.

Data sharing statement provided by the authors is available with the full text of this article at jebmh.com.
Financial or other competing interests: None.
Disclosure forms provided by the authors are available with the full text of this article at jebmh.com.

REFERENCES

[1] Obladen M. Necrotizing enterocolitis – 150 years of fruitless search of the cause. Neonatology 2009;96(4):203-210.
[2] Thomas C, Krishan L. Neonatal necrotizing enterocolitis. Indian Pediatr 1997;34(1):47-51.
[3] Rodin AE, Nichols MM, Hsu FL. Necrotizing enterocolitis occurring in full-term neonates at birth. Arch Pathol 1973;96(5):335-338.
[4] Maayan-Metzger A, Itzchak A, Mazkereth R, et al. Necrotizing enterocolitis in full-term infants: case–control study and review of the literature. J Perinatol 2004;24(8):494-499 .

[5] Schmidt K. Über eine besonders schwer verlaufende Form von Enteritis beim Säugling, 'Enterocolitis ulcerosa necroticans'. I. Pathologisch-anatomische Studien. Oesterr Z Kinderheilkd 1952;8:114-136.
[6] Berdon WE, Grossman H, Baker DH, et al. Necrotising enterocolitis in the premature infant. Radiology 1964;83:879-887.
[7] Walsh MC, Kliegman RM. Necrotizing enterocolitis: treatment based on staging criteria. Pediatr Clin North Am 1986;33(1):179-201.
[8] Kliegman RM, Walsh MC. Neonatal necrotizing enterocolitis: pathogenesis, classification and spectrum of illness. Curr Probl Pediatr 1987;17(4):213-288.
[9] Caplan MS, Kelly A, Hsueh W. Endotoxin and hypoxia-induced intestinal necrosis in rats: the role of platelet activating factor. Pediatr Res 1992;31(5):428-434.
[10] Narang A, Rao R, Bhakoo ON. Neonatal necrotizing enterocolitis: as epidemiological study. Indian Pediatr 1993;30(10):1207-1214.
[11] Kliegman RM, Fanaroff AA. Neonatal necrotizing enterocolitis: a nine year experience. I. Epidemiology and uncommon observations. Am J Dis Child 1981;135(7):603-607.
[12] Flidel-Rimon O, Branski D, Shinwell ES. The fear of necrotizing enterocolitis versus achieving optimal growth in preterm infants - an opinion. Acta Paediatr 2006;95(11):1341-1344.
[13] Ballance WA, Dahms BB, Shenker N, et al. Pathology of neonatal necrotizing enterocolitis. J Pediatr 1990;117(1 Pt 2):S6-S13.
[14] Gupta S, Morris JG Jr, Panigrahi P, et al. Endemic enterocolitis: lack of association with a specific infectious agent. Pediatr Infect Dis J 1994;13(8):728-734.
[15] Holl K, Nolte H, Zornig C, et al. Pneumatosis intestinalis--histology, immunocytochemistry and new theory of morphogenesis. Der Pathologe 1993;14(4):199-204.
[16] Rotbart HA, Nelson WL, Glode MP, et al. Neonatal rotavirus-associated necrotizing enterocolitis: case control study and prospective surveillance during an outbreak. J Pediatr 1988;112(1):87-93.
[17] Jilling T, Lu J, Jackson M, et al. Intestinal epithelial apoptosis initiates gross bowel necrosis in an experimental rat model of neonatal necrotizing enterocolitis. Pediatr Res 2004;55(4):622-629.