ORIGINAL ARTICLE

Risk factors of necrotising enterocolitis among 28-34 weeks preterm neonates at a Tertiary Care Hospital, East Java, Indonesia

Brigitta I.R.V. Corebima, MD[1,2], Prof. Kusworini Handono[3], Dr. Wisnu Barlianto[2], Dewi Santosaningsih, PhD[4], Prof. Rinawati Rohsiswatmo[5], Eko Sulistijono, MD[2], Widanto, MD[6], Indrastuti Normahayu, MD[7]

[1]Doctoral Programme of Medical Science, Faculty of Medicine, Universitas Brawijaya, Malang, East Java, Indonesia, [2]Department of Paediatrics, Dr. Saiful Anwar General Hospital-Faculty of Medicine, Universitas Brawijaya, Malang, East Java, Indonesia, [3]Department of Clinical Pathology, Dr. Saiful Anwar General Hospital-Faculty of Medicine, Universitas Brawijaya, Malang, East Java, Indonesia, [4]Department of Clinical Microbiology, Dr. Saiful Anwar General Hospital-Faculty of Medicine, Universitas Brawijaya, Malang, East Java, Indonesia, [5]Department of Paediatrics, Dr. Cipto Mangunkusumo General Hospital-Faculty of Medicine, University of Indonesia, Jakarta, Indonesia, [6]Department of Paediatric Surgery, Dr. Saiful Anwar General Hospital-Faculty of Medicine, Universitas Brawijaya, Malang, East Java, Indonesia, [7]Department of Radiology, Dr. Saiful Anwar General Hospital-Faculty of Medicine, Universitas Brawijaya, Malang, East Java, Indonesia

ABSTRACT

Introduction: Necrotising enterocolitis (NEC) is a serious health problem primarily affects preterm and very low birthweight (VLBW) infants. However, the pathomechanism of NEC remains elusive. This study aimed to analyse the risk factors for NEC among preterm neonates in East Java, Indonesia.

Materials and Methods: A single-centre, prospective, case-control study involving 32 subjects of preterm neonates was conducted at a tertiary care hospital in Malang, East Java, Indonesia between January to June 2022. A total of 15 preterm neonates with NEC and 17 preterm neonates without NEC were enrolled in this study. Data on demographic, clinical and laboratory findings were collected. Multiple logistic regression test was performed to analyse the risk factors for NEC development. Further profiling within 15 subjects with NEC, i.e., NEC grade ≥ II, were conducted to collect systemic, abdominal, laboratory, abdominal x-ray (AXR) and blood culture findings.

Results: The risk factors related to NEC development in preterm infants were multi-morbidity (adjusted OR = 11.96; 95% CI 1.85 168.38; p = 0.046), antibiotic exposure (OR = 15.95; 95% CI 1.54 165.08; p = 0.020) and requiring advanced neonatal resuscitation at birth (OR = 10.04; 95% CI 1.09 92.11; p = 0.041). Further profiling within NEC cohorts highlighted respiratory distress (86.7%), (oro)gastric retention (80.0%), thrombocytopenia (53.3%), gastrointestinal dilatation in AXR (53.3%), and positive blood culture *Klebsiella pneumoniae* (40.0%) were most common findings.

Conclusion: Preterm neonates with multimorbidity, prolonged antibiotic exposure, and requiring advanced resuscitation at birth were more likely to develop NEC. Early detection of the risk factors and determinant factors for survival may help to improve the clinical outcome.

KEYWORDS:
Clinical characteristics, necrotising enterocolitis, prematurity, risk factors

INTRODUCTION

Necrotising enterocolitis (NEC) is an acute inflammatory, multifactorial disease of intestinal injury and necrosis which primarily affect preterm infants and is a leading cause of morbidity and mortality.[1] The global incidence of NEC is reported to vary from 7 to 13% in preterm and very low birthweight (VLBW) infants, i.e., birthweight < 1500 gm.[2,3] In Indonesia, the incidence was 8.6% among preterm infants born in single-centre tertiary hospital, yet the data were sparse and limited.[4] Of all NEC cases primarily in VLBW infants, 27 to 52% was reported to require further surgical intervention, including laparotomy and bowel resection.[5] Despite modern advances in intensive neonatal care, NEC mortality rate remains frequent, with rates reported between 18% and 63%.[6–8]

Although the aetiology of NEC remains elusive, multiple risk factors, including prematurity, low birth weight, hypoxia, abnormal microbiota colonisation in the intestinal tract, microcirculatory disorders, formula feeding and patent ductus arteriosus, were highlighted to involve in the development of NEC.[9] Recent studies showed sepsis, as a severe infectious disease, is considered a contributing factor for NEC. The incidence of NEC in sepsis patients ranges from 34% to 57%.[10,11] However, these risk factors findings might be widely varied based on advancements of neonatal intensive care that might differ between developed and developing country, leading to a varying degree of neonatal risk factors in NEC development. In all regards, NEC becomes one of the most devastating conditions in preterm and VLBW infants.

Diagnosis of NEC is established by Modified Bell's criteria, which comprises of systemic signs, abdominal and radiologic findings.[12] Due to the multifactorial nature of NEC, clinical

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Corresponding Author: Brigitta I.R.V. Corebima
Email: brigitta_vebi@yahoo.com; briggita_vebi@ub.ac.id

manifestations were non-specific and overlapping with other disease of gastrointestinal origins; clinicians thus depend on plain abdominal x-ray (AXR) to find the telltale sign of NEC, i.e. pneumatosis intestinalis, where it was not always present and approximately found in 39.4% of all NEC cases.[13–15] Therefore, identifying specific risk factors for NEC in preterm infants would be helpful to optimise strategies to reduce morbidity and mortality, as well as to provide directions for clinical treatment strategies. This present study was aimed to describe the incidence, characteristics, and determine the correlation between risk factors of NEC for preterm neonates in a tertiary hospital in Malang, East Java province of Indonesia. Further profiling of abdominal signs, AXR and laboratory findings within our NEC cohorts might contribute to a description that may differ in other country.

MATERIALS AND METHODS

Study design and patients

This case-control study, involving subjects of preterm neonates born at a gestational age (GA) < 35 weeks, was conducted at a single-centre tertiary hospital perinatology unit of Saiful Anwar General Hospital (SAGH), East Java, Indonesia, between January to June 2022. The subjects were followed-up and further divided into two groups, i.e., preterm neonates with NEC and preterm neonates without NEC. Subjects were followed-up until reaching clinical outcomes or age of 28 days. All subjects included in this study were received exclusive or predominant breastfeeding and none of them received probiotics. This study was approved by Research Ethics Committee of Saiful Anwar General Hospital and registered to clinicaltrials.gov (NCT05335577) as part of more comprehensive study. Informed consents were obtained from all parents.

Extensive data on GA, birthweight, sex, singleton, Ballard score of maturity, Lubchenco intrauterine growth criteria, Downes score (DS) of respiratory distress, APGAR score, and extent of neonatal resuscitation was obtained. Additional clinical data included surfactant administration, antenatal corticosteroid, history of packed red cells (PRC) transfusion, clinical pathologies, antibiotic exposure and laboratory findings were also collected. Maternal profiles of comorbidities, body mass index, mode of delivery and passive smoker status were collected. These data were then analysed to be included in risk factor analysis.

Among NEC cohorts detected in this study, further profiling was conducted on systemic signs, abdominal, laboratory and AXR findings based on Modified Bell's criteria. In NEC cohorts developing sepsis, blood culture results were also collected.
Data collection was ceased in case of death, transfer to another hospital or parents willing to drop out of study. Preterm infants were excluded in case of severe congenital anomalies, surgery within study period, death before the diagnosis of NEC established or parents' refusal upon informed consent.

Definitions

NEC cases were defined as infants diagnosed with NEC grade 2 or higher based on Modified Bells' criteria. NEC cases were established by two independent neonatologists. AXR features of cohorts were reviewed independently by one senior radiologist blinded to the study. Blood samples and laboratory features were analysed independently by central clinical pathology unit of SAGH.

Neonatal resuscitation was, based on Indonesian Pediatric Association national protocol, adopted from Neonatal Resuscitation Program 8th edition.[16,17] There were four main steps of neonatal resuscitation based on severity, i.e., initial steps (warm, dry, stimulate and position airway), followed by positive-pressure ventilation (PPV), cardiac compression and intubation and epinephrine administration at birth. Advanced resuscitation was defined as neonates requiring PPV or higher intervention of resuscitation, i.e., cardiac compression and intubation, or epinephrine administration at birth.

Surfactant administration was defined as exogenous surfactant replacement therapy protocol given within 24-h of life. Antenatal corticosteroid administration was defined as intravenous corticosteroid given within 24 to 48-h before birth in part of lung maturation induction. History of PRC transfusion was defined as red blood cells transfusion within 48-h prior to NEC development or within 14 days of life. Exposure to antibiotics were defined as antibiotic administration within first week of life or minimum ≥ 48-h prior to NEC diagnosis. The type of antibiotics administered were also noted.

Clinical multimorbidity was defined as diagnoses established by the attending neonatologist in response to patients' conditions. Multiple morbidities were defined as subjects suffered from > 2 co-occurring clinical diagnoses. Sepsis was defined as positive blood culture confirming bacteraemia or procalcitonin level > 2.0 ng/ml with corresponding clinical pictures of temperature instability and bradycardia. Severe congenital anomalies were defined as confirmed congenital abnormalities that lead to hemodynamic instability, i.e., hypoperfusion or shock. Surgery exclusion criteria was defined as any surgery within study period at indication other than NEC grade 3.

Statistical analysis

This present study performed multistep analysis on the cohort. During the study period, the demographic, clinical and laboratory parameters of all preterm infants with NEC were compared with those preterm infants without NEC. A descriptive analysis was conducted to obtain central tendencies of the cohorts; numeric variables was expressed in mean ± SD or median and interquartile range (IQR), while categorical variables expressed in frequency and percentages. Predictive factors with p values of ≤ 0.25 were included in the multiple logistic regression analysis using backward stepwise selection method.

RESULTS

Overall cohort

A total of 35 subjects were recruited, however three subjects from preterm non-NEC dropped out due to guardian's refusal to participate in this study. Among them, 15 (10.07%, 15/149 subjects) preterm infants developed NEC and 17 preterm non-

Table I: Demographic and clinical characteristics of subjects at birth

Characteristics	Value
Neonatal factors	
Gestational age (weeks, median, IQR)	31.5 (30.5 34.0)
Birthweight (grams, mean ± SD)	1562.81 ± 330.23
Sex (n, %)	
Male	11 (34.4)
Female	21 (65.6)
APGAR score at 1' (median, IQR)	6 (3-6)
APGAR score at 5' (median, IQR)	8 (5-8)
Lubchenco intrauterine growth criteria (n, %)	
Small for gestational age (SGA)	6 (18.8)
Appropriate for gestational age (AGA)	26 (81.3)
Downes score at birth (median, IQR)	3 (0-4)
Antenatal corticosteroid (n, %)	
No	23 (71.9)
Yes	9 (28.1)
Nutritional type (n, %)	
Breastmilk	25 (78.1)
Predominant breastmilk	7 (21.9)
Laboratory aspects at birth (mean ± SD)	
Haemoglobin (g/dl)	15.78 ± 2.28
Leukocyte count (cell/mm^3)	11 963 ± 5 934
Platelets count (cell/mm^3)	235 927 ± 70 491
Haematocrit (%)	45.85 ± 7.05
Absolute neutrophil count (cell/mm^3)	6 213 ± 4 456
Immature/total neutrophil ratio	0.19 ± 0.51
C-reactive protein (mg/l)	0.207 ± 0.44
Random blood glucose (mg/dl)	75.16 ± 22.70
Maternal factors	
Singleton vs twin pregnancy (n, %)	
Singleton	25 (78.1)
Gemelli (twin)	7 (21.9)
Mode of delivery (n, %)	
Pervaginam	8 (25.0)
Caesarian section	24 (75.0)
Body Mass Index (BMI) (n, %)	
18-25 kg/m^2	30 (93.8)
< 18 kg/m^2 or > 25 kg/m^2	2 (6.2)

APGAR score = Appearance, pulse, grimace, activity, respiratory score; IQR = Interquartile range (25th – 75th quartile); SD = Standard deviation.

NEC were eligible to participate in this study. During the study period, all preterms with NEC participated. Subjects with NEC (n = 15) were comprised of five males (33.3%), while preterm without NEC were consisted of six males (35.3%) with no significant difference was found between the two groups (p = 0.907, OR 0.92 95% CI 0.21 3.96). The overview of demographic, clinical, and laboratory features is summarized in Table I.

Case (NEC) and control cohorts

Univariate analysis showed the risk factors for NEC development including GA < 32 weeks (OR = 13.00; 95% CI 2.40 70.46; p = 0.001), birthweight < 1500 grams (OR = 8.94; 95% CI 1.80 44.34; p = 0.005), DS of neonatal respiratory distress ≥ 4 (OR = 20.63; 95% CI 3.19 133.4; p < 0.001), requirements of advanced neonatal resuscitation upon birth (OR =1 8.00; 95%CI 2.76 117.6; p = 0.001), and history of PRC transfusion (OR = 2.70; 95% CI 1.65 4.42; p = 0.010), multiple morbidities (OR = 45.5; 95% CI 4.48 461.9; p < 0.001), and history of antibiotic exposure (OR = 48.75; 95% CI 5.99 396.5; p < 001). Several factors contributed statistically significant in the odds of preterm neonates developing NEC was depicted in Table II. Furthermore, outcome of adjusted odds ratio through multivariate analysis were summarized in Table III.

Preterm with NEC cohort

Preterm neonates with NEC were developed in 15 subjects (47%) with incidence of 10.07%. This cohort was analysed further and described based on Modified Bell's criteria parameters. Signs of shock were defined as decrease of tissue perfusion characterised by cold clammy extremities, capillary refill time (CRT) > 2 s, or hypotension requiring inotropes or vasopressor. Neonates that developed sepsis clinically were confirmed by positive blood culture. Early onset NEC were defined as onset of NEC less than or equal to 7 days of life.[18] In this study, 66.7% of cases were early-onset, with a median age of diagnosis of 5 days. The most prominent aetiology found in blood culture was *Klebsiella pneumoniae*. Table IV summarised the clinical finding on preterm neonate subjects with NEC.

Subjects with NEC were 14 (93.3%) had multiple clinical pathologies with the most prominent were neonatal pneumonia (12 subject, 80%) that developed respiratory failure (11 subject, 73.3%) and hyaline membrane disease (9 subject, 60%). The clinical pathologies other than NEC was depicted in Table V. Transfusion of PRC was found significantly higher in NEC cohort with the average dose of 27.32 ml/kg. Antibiotic exposure was correlated with higher

Table II: Contributing risk factors related to NEC development between subjects

Parameters	Preterm with NEC	Preterm without NEC (Control)	p-value	OR (95% CI)
Total n = 32 (100%)	15 (47%)	17 (53%)		
	NEONATAL FACTORS			
Gestational age (%)				
< 32 weeks	12 (80)	4 (23.5)	**0.001***[a]	13.00 (2.40 70.46)
≥ 32 weeks	3 (20)	13 (76.5)		
Birthweight (%)				
< 1500 gram	11 (73.3)	4 (23.5)	**0.005***[a]	8.94 (1.80 44.34)
≥ 1500 gram	4 (26.7)	13 (76.5)		
Downes score criteria				
≥ 4	11 (73.3)	2 (11.8)	**< 0.001***[a]	20.63 (3.19 133.4)
< 4	4 (26.7)	15 (88.2)		
APGAR score at 1' criteria				
0-3	5 (33.3)	4 (23.5)	0.538[a]	1.63 (0.34 7.67)
4-6	10 (66.7)	13 (76.5)		
APGAR score 5'				
0-6	6 (40.0)	4 (23.5)	0.316[a]	2.17 (0.47 9.95)
7-10	9 (60.0)	13 (76.5)		
Extent of neonatal resuscitation (%)				
Positive-pressure ventilation	12 (80.0)	4 (23.5)		Reference
Cardiac compression and intubation	1 (6.7)	1 (5.9)	0.457	3.00 (0.15 25.9)
No indication of resuscitation	2 (13.3)	12 (70.6)	**0.001***[a]	18.00 (2.76 117.6)
Lubchenco criteria (n, %)				
Small for gestational age (SGA)	1 (6.67)	6 (35.8)	**0.011***[a]	2.36 (1.51 3.70)
Appropriate for gestational age (AGA)	14 (93.3)	11 (64.7)		
Surfactant administration (%)				
No	13 (86.7)	17 (100.0)	0.120[a]	0.43 (0.29 0.65)
Yes	2 (13.3)	0		
History of PRC transfusion (%)				
Yes	5 (33.3)	0	**0.010***[a]	2.70 (1.65 4.42)
No	10 (66.7)	17 (100.0)		
Sepsis				
Yes	11 (78.6)	1 (5.9)	**< 0.001***[a]	88.0 (7.08 139.9)
No	3 (21.4)	16 (94.1)		
Multimorbidity (%)				
Yes	14 (93.3)	4 (23.5)	**< 0.001***[a]	45.5 (4.48 461.9)
No	1 (6.7)	13 (76.5)		
Antibiotic exposure (%)				
Yes	13 (86.7)	2 (11.8)	**< 0.001***[a]	48.75 (5.99 396.5)
No	2 (13.3)	15 (88.2)		
Clinical outcome (%)				
Death	12 (80.0)	1 (5.9)	**< 0.001***[a]	64.0 (5.90 694.1)
Discharged	3 (20.0)	16 (94.1)		
Length of stay (days)	15 (11-22)	8 (5-10.5)	**0.001***[b]	
	Maternal factor			
Maternal comorbidities (%)				
Any comorbidity	14 (93.3)	14 (82.4)	0.087[a]	2.07 (1.42 3.02)
No comorbidity	1 (6.7)	3 (17.6)		
Maternal comorbidities (%)	0 (0)	2 (11.8)		Reference
No comorbidity	8 (53.3)	7 (41.2)	0.156	2.14 (1.35-3.68)
PPROM, PROM	2 (13.3)	3 (17.6)	0.290	1.67(0.82-3.41)
Pre-eclampsia/eclampsia	1 (6.7)	2 (11.8)	0.361	1.50(0.67-3.34)
Antepartum bleeding	5 (26.7)	3 (17.6)	0.151	2.33 (0.99-5.49)
Others				
Amniotic fluid index criteria (%)				
Oligohydramnios	3 (23.1)	3 (20.0)	0.843[a]	1.20 (0.20-7.31)
Normal AFI	10 (76.9)	12 (80.0)		
Pre-partum antibiotic (%)				
No	1 (6.7)	5 (29.4)	0.100[a]	0.171 (0.02-1.68)
Yes	14 (93.3)	12 (70.6)		
Passive smoker status (%)				
No	9 (60.0)	6 (35.3)	0.210[a]	0.640 (0.31-1.32)
Yes	6 (40.0)	10 (63.5)		

[a]Analysis with Chi-square test; [b]Analysis with independent t-test; *p-value < 0.05 was considered statistically significant. APGAR score = Appearance, Pulse, Grimace, Activity, Respiratory score. CI = confidence interval. NEC = necrotising enterocolitis. OR = odds ratio, crude. PPROM = Premature prelabour rupture of the membrane. PROM = Prelabour rupture of the membrane. PRC = Packed red blood cells.

Table III: Multivariate analysis of risk factors related to NEC

Parameters	Preterm with NEC	Preterm without NEC (Control)	p-value	Adjusted OR (95% CI)
Total n = 32 (100%)	15 (47%)	17 (53%)		
Multimorbidity (%)				
Yes	14 (93.3)	4 (23.5)	0.046*	11.95 (1.85 168.38)
No	1 (6.7)	13 (76.5)		
Antibiotic exposure (%)				
Yes	13 (86.7)	2 (11.8)	0.020*	15.95 (1.54 165.08)
No	2 (13.3)	15 (88.2)		
Extent of neonatal resuscitation (%)				
Requiring advanced resuscitation	13 (86.7)	5 (29.4)	0.041*	10.04 (1.09 92.11)
No indication of resuscitation	2 (13.3)	12 (70.6)		

CI = Confidence interval. OR = odds ratio. NEC = Necrotising enterocolitis.

Table IV: Clinical presentations upon NEC diagnosis

Systemic clinical signs	**N (%)**
Respiratory distress	13 (86.7)
Signs of shock	8 (53.5)
Temperature instability	7 (47.6)
Apnea	3 (20.0)
Bradycardia	1 (6.7)
Abdominal clinical signs	**N (%)**
Gastric retention	12 (80.0)
Gastrointestinal bleeding	9 (60.0)
Abdominal distension	8 (53.3)
Decreased bowel sound	1 (6.7)
Heme-positive stool	1 (6.7)
Laboratory findings	**N (%)**
Thrombocytopenia	8 (53.3)
Metabolic acidosis	7 (46.7)
Disseminated intravascular coagulopathy	3 (20.0)
Plain abdominal radiologic features	**N (%)**
Gastric and/or intestinal dilatation	8 (53.3)
Intestinal wall thickening	1 (6.7)
Pneumatosis intestinalis	3 (20.0)
Pneumoperitoneum	1 (6.7)
Blood culture results	**N (%)**
Klebsiella pneumoniae	6 (40.0)
Pseudomonas aeruginosa	1 (6.67)
Enterococcus faecium	1 (6.67)
Aeromonas salmonicida	1 (6.67)
No colony growth was found	6 (40.0)
Modified Bell's criteria	**N (%)**
NEC grade II	13 (86.7)
NEC grade III	2 (13.3)
Age of NEC diagnosis (days) (median, IQR)	5 (3-9)
Onset of NEC	**N (%)**
Early-onset	10 (66.7)
Late-onset	5 (33.3)

IQR = Interquartile range (25th – 75th quartile); NEC = Necrotising enterocolitis.

odds of developing NEC. In this study, the most frequently administered antibiotics prior to NEC diagnosis were ampicillin-sulbactam and gentamycin (n = 13, 86.7%) and followed by amikacin (n = 3, 20%) and meropenem (n = 2, 13.3%).

DISCUSSION

NEC is an acute, multifactorial inflammatory disease of intestinal injury which primarily affect preterm infants. The exact aetiology and pathophysiology of NEC remained elusive because of its multifactorial nature. Various risk factors involvements had been proposed, including both neonatal and maternal-related factors.[2,9] However, the most consistent risk factors remained prematurity and birthweight, the increasing prematurity and the lesser birthweight increased the odds of the infants to develop NEC.[2,11] This study confirmed, once again, that prematurity and lower birthweight were consistent risk factors of NEC.

The incidence rate of NEC among preterm infants in this study was 10.07%. This finding was higher than those from other countries. Zozaya et al (2022) highlighted 8.8% NEC incidence in Spain preterm cohorts.[19] Considering ethnicity

Table V: Clinical pathologies other than necrotising enterocolitis

Clinical pathologies	N (%)
Neonatal pneumonia	12 (80.0)
Respiratory failure	11 (73.3)
Hyaline membrane disease	9 (60.0)
Septic shock	9 (60.0)
Indirect hyperbilirubinemia	8 (53.5)
Early onset sepsis	7 (46.7)
Late onset sepsis	5 (33.3)
Vitamin D deficiency	3 (20.0)
Acyanotic congenital heart disease	2 (13.3)
Vitamin K deficiency related bleeding	2 (13.3)

factors, the incidence of NEC was 9 to10% according to Malaysian National Neonatal Registry (MNNR) 2004 to 2005 where this study involved VLBW infants, where most preterm infants manifested.[20] This study were also showed a higher incidence compared to similar single-centre study in Jakarta, Indonesia, where the incidence were 8.6% among preterm infants.[4] The varying incidence of NEC might be attributable to different degree of clinical comorbidities experienced by subjects. The advances in neonatal intensive care were correlated with longer survival of severely distressed neonates and this might contribute to increasing diagnosis of NEC in our cohorts.

Contributing risk factors in the diagnosis of NEC within preterm infants in this study were GA, birthweight, DS of respiratory distress, requirement of advanced neonatal resuscitation, history of PRC transfusion within 48-h prior to diagnosis, multiple clinical pathologies, and antibiotics exposure. This study confirmed prematurity and VLBW were consistent risk factors attributable to NEC regardless of ethnicity or difference in country income status.[2,19,21]

More recent pathogenesis showed that prematurity exposed infants to a higher odd of NEC was thought to be gut dysbiosis. Previous studies noted that there was significant difference in intestinal microbiota between full-term and preterm neonates. The GA was proposed as the pivotal driver of the premature gut microbiota establishment, as prematurity is believed to be attributable to a delay in the transition to an established adult-type signature microbiota. This notion supported that there is a significant reduction in the incidence of NEC within full-term neonates, as their gut microbiota were more likely to be similar to adult-type.[22–24] In preterm infants, there is increased relative abundance of Proteobacteria, with some of its member were Enterobacteriaceae, *Klebsiella spp* and *E. coli*, followed by a decrease in Firmicutes (*Lactobacillus spp*) and Bacteroidetes (*Bacteroidetes spp* and *Prevotella spp*).[22,23] Previous study conducted by Corebima et al (2019) also exhibited the role of increasing *Klebsiella pneumoniae* in preterm infant microbiome was correlated with a higher degree of human β-defensin 2 levels where it posed significant higher inflammatory response within gut mucosal microenvironment.[25] These previous findings showed preterm infants exhibited delayed establishment of commensal anaerobic microbes compared to their full-term counterparts and might explain some possible routes why, in this study, the isolated blood-borne bacteria was *Klebsiella pneumoniae* in our cohorts. However, further investigation is encouraged to confirm gut dysbiosis and NEC, especially in Indonesia population.

In this study, no probiotics were given in both of our cohorts. The role of probiotics was debatable, but the proposed mechanism was modulating gut microbiota. However, the role of probiotics was still limited as most strains were under investigation in small experimental population. A retrospective cohort study by Que et al (2021) showed no significant difference in the incidence and severity of NEC within 310 subjects receiving *Bifidobaterium* and *Lactobacillus* probiotics vs 355 that did not, i.e. (4% vs 5%, p = 0.10).[26] A phase 3 trial involving 1314 preterm infants showed evidence of benefit for probiotics intervention for both NEC or late-onset sepsis finding; thus routine administration of probiotics was not recommended.[27] However, a number of clinical trials were conducted to confirm debatable findings of probiotics in preventing NEC.

The need of advanced neonatal resuscitation at birth, i.e., requirement of PPV or higher, at birth was correlated with higher odds of developing NEC and further strengthen its impact after conduction of multivariate analysis. Advanced resuscitation was significantly found in NEC, most commonly positive-pressure ventilation, in contrast with its non-NEC counterpart where resuscitation was not indicated or only needed initial steps of resuscitation. This finding was correlated with the degree of hypoxemia and respiratory distress found in this study, i.e., D). DS was assessed by following parameters, i.e., respiratory rate, cyanosis, retraction, grunting and respiratory sound, to clinically evaluate the degree of respiratory distress in infants, where DS < 4 meant mild respiratory distress and ≥ 4 were moderate-to-severe distress.[28] The subjects' DS was assessed within 30 to 60' postnatal.

Both advanced neonatal resuscitation and degree of respiratory distress were correlated with systemic hypoxia/ischaemia, where it was further validated with the source of hypoxic insult due to hyaline membrane disease and/or neonatal pneumonia in our study. The other signs of hypoxia were noted by lower APGAR score at 1' and 5', although did not reach statistical significance, it showed overall lower APGAR score in NEC cohort. Systemic hypoxic/ischemic insult was attributable to NEC development. In a recent study by der Heide et al (2020), hypoxic/ischemic event was correlated with higher risk of developing NEC as this hypoxic event might cause splanchnic hypoperfusion leading to gut mucosal ischemia and mucosal integrity disruption.[29,30]

PRC transfusion was correlated with higher odds of developing NEC. This is an interesting finding since it is a relatively recent discovery associated with the transfusion-associated NEC (TANEC). Limited studies were found to correlate PRC transfusion with the risk of NEC. TANEC has been described as NEC that arises within 48 hours following blood transfusion.[31] A review by Gephart (2012) showed PRC transfusion was related to NEC, the hypothesised mechanisms were the result of an abnormal response of the mesenteric blood flow velocity (MBFV) in the post-transfusion state such that low perfusion state interacted with the mechanism of feeding and contribute to intestinal injury. In that sense, two other studies showed practice to hold feeding before and during transfusion was correlated with the decreased of TANEC incidence in preterm infants.[31–33] However, another finding from a prospective, multicentre cohort exhibited exposure to RBC transfusion was not correlated with increased risk of NEC, but the hypoxic insults did, i.e., anaemia as the underlying condition that contribute to NEC.[34] Another proposed pathophysiology of transfusion and NEC was iron overload, confirmed by serum ferritin assay. However, the exact cutoff between transfusion and higher risk of NEC was limited. As there were limited subjects of NEC with transfusion in our study period, an investigation to analyse the correlation between PRC transfusion dosage and NEC is needed.

In multivariable analysis, multiple clinical pathologies and antibiotic exposure were attributed to the higher risk of NEC in this study. Clinical pathologies were associated with systemic hypoxic/ischemic hits and therefore increase the risk of NEC. In a longitudinal multicentre cohorts of preterm infants showed that comorbidities were attributed to higher risk of NEC, where the more severe pathologies was correlated to a higher degree of ischemic insults in GI mucosal integrity leading to inflammation and necrosis.[19,29]

Among multiple factors associated with pathogenesis of NEC, the widespread administration of antibiotics in NICU might play role in the development of NEC in preterm infants. Although the use of antibiotics was targeted to combat systemic infection, an adverse effects of antibiotic use in infants' immature GI tract was hypothesised. This finding was in concordance with other studies. As gut microbiome in preterm infants differed from their full-term counterparts, antibiotic exposure, especially prolonged administration, were correlated with a higher degree of reducing infants gut microbiota diversity and promoting overgrowth of pathogenic microbes over commensal species, such as Enterobacteriaceae and Clostridia.[22,35] As the commencement of antibiotics is within neonatologist jurisdiction, a more strict risk-to-benefit ratio is required and the need of consensus establishment of evidence-based duration of antibiotic for neonatal sepsis is essential.

This study was conducted in a prospective manner, allowing multiple risk factors to be evaluated simultaneously to get outcome of interest longitudinally in an exact timestamp. Any confounding was mitigated with strict inclusion criteria, stratification, and multivariate analysis. However, there was some limitation of this study due to its limited number of subjects and single-centre setting. Further investigation is encouraged to validate these findings in a larger, multi-centre inter-hospital cohort and randomised trials.

CONCLUSION

This prospective, case-control study confirmed that lower gestational age, VLBW, degree of respiratory distress and neonatal resuscitation at birth were correlated with higher risk of developing NEC in preterm neonates. Other factors contribute to higher odds of NEC was administration of PRC transfusion within 48-h prior to diagnosis of NEC. From multivariate analysis, multimorbidity, prolonged antibiotic exposure, and the extent of neonatal resuscitation were independently correlated with higher risk of NEC development. Further researches are required to validate these findings with larger, multicentre study.

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CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

ETHICAL APPROVAL

The study had been registered with the local ethics committee under Saiful Anwar General Hospital, Health Research Ethics Commission no. 400/011/K.3/302/2022. Informed consents were obtained from all parents.

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AUTHORS CONTRIBUTIONS

BIRVC: Conceptualisation, methodology, investigation, data curation, writing – original draft. KH: Validation, formal analysis, resources, writing – review and editing. WB: Funding acquisition, project administration, visualisation, writing – original draft. DS: Validation, writing – review and editing, data curation. RR: Supervision, validation, methodology. ES: Data curation, investigation, formal analysis. WW: Formal analysis, investigation, resources. IN: Validation, data curation, visualisation.

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