HAYDARPAŞA NUMUNE MEDICAL JOURNAL

DOI: 10.14744/hnhj.2023.74555
Haydarpasa Numune Med J 2024;64(2):187–192

ORIGINAL ARTICLE

Risk Factors of Necrotizing Enterocolitis in Preterm Infants: A Single Center Experience

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Abstract

Introduction: Necrotizing enterocolitis (NEC) is a fatal disease with up to 20% mortality rates. Identifying risk factors for NEC may reduce NEC incidences. This study aims to investigate NEC-related risk factors in preterm infants.

Methods: We included 27 preterm infants with NEC and 35 infants without NEC among the preterm newborns (n=1669) hospitalized in the Duzce University School of Medicine neonatal intensive care unit between 2009 and 2021. Parametric numeric data were calculated using the independent sample's t-test. Two-sample comparisons of nonparametric data were performed using the Mann-Whitney test. Pearson chi-square, Yates correction, and Fisher's exact test were also used to evaluate the categorical data.

Results: Our results agree with previous studies regarding some of these findings: birth weight is lower in the NEC group (1.37±0.49 kg) than in the non-NEC group (18.3±6.5 kg) (p=0.009), with statistically similar gestational age. We couldn't show the association between NEC and multiple gestations, chorioamnionitis, preeclampsia, Apgar scores, patent ductus arteriosus, mechanical ventilation, pre-NEC red blood cell, or fresh frozen plasma transfusions. In the NEC group, thrombocyte levels before NEC were significantly lower (98 [9-2253]) (/x10^3 mm^3) than in the control group (222 [17-345]) (/x10^3 mm^3) (p=0.012). In addition, mortality rates (22.2% vs. 2.9%, respectively) (p=0.037), use of vasopressors (29.6% vs. 2.9%, respectively) (p=0.008) were markedly higher in the NEC group than in the non-NEC group. Additionally, lower birth weight (NEC group: 1367.25±493.62 vs. non-NEC group: 1831.71±651.62) (p=0.009), prolonged use of antibiotics (NEC group: 24% vs. non-NEC group: 0%) (p=0.004), and poor circulation (NEC group: 84% vs. non-NEC group: 3%) (p<0.001) were statistically significant variables.

Discussion and Conclusion: NEC increases the mortality rates in preterm infants. The use of vasopressors, low birth weight, poor circulation, and antibiotics are significant risk factors for NEC, and low thrombocyte levels can lead to the prediction of NEC.

Keywords: Infant premature; necrotizing enterocolitis; risk factors.

Necrotizing enterocolitis (NEC) is a mortal gastrointestinal emergency, presenting with abdominal distention, gastric residuals, and rectal bleeding[1]. The pathophysiology of NEC is uncertain and is suspected to be multi-factorial. Unfortunately, there is not enough data about genetic predisposition[2].

Experts conclude that low birth weight, prematurity, and feeding with formula are associated with NEC. However, other risk factors are controversial. For example, several studies found that intrauterine growth retardation, severe anemia, and erythrocyte transfusion 48 hours before necrotizing enterocolitis were associated with an increased risk for NEC. Fresh frozen plasma was also a potential risk factor for NEC in a study, probably due to the more viscous component of donor plasma obtained from adults than from neonates. In addition, natal and postnatal risk factors

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Submitted Date: 10.12.2022 **Revised Date:** 24.03.2023 **Accepted Date:** 05.04.2023
Haydarpaşa Numune Medical Journal

are associated with asphyxia histories, low birth weight, sepsis, patent ductus arteriosus, congenital heart disease, mechanical ventilation, starvation, and use of vasopressors. However, in several studies, cesarean section, breastfeeding, and surfactant treatment were protective against NEC[3,4].

A recently published meta-analysis revealed maternal gestational diabetes mellitus, intrahepatic cholestasis in pregnancy, preeclampsia, neonatal asphyxia, sepsis, patent ductus arteriosus, congenital heart disease, use of antibiotics, and vasopressors were statistically significant risk factors. However, transfusion, mechanical ventilation, and anemia showed considerable heterogeneity[5].

The current study aimed to further explore the influence of risk factors on NEC by comparing preterm infants with NEC and without NEC.

Materials and Methods

We designed this retrospective study, including the newborns hospitalized in the Duzce University School of Medicine neonatal intensive care unit between 2009 and 2021. This study included 27 preterm infants with NEC and 35 infants without NEC. The Duzce University School of Medicine Ethics Committee approved the study (approval number 2022/85). The study was performed under the principles of the Helsinki Declaration. Therefore, the preterm neonates who died within seven days of life were not included in the non-NEC group. The diagnoses of NEC were confirmed according to Bell's criteria with stage ≥2.

According to the Modified Bell Staging criteria, stage I is a suspected NEC with non-specific symptoms and abdominal signs. In the presence of grossly bloody stools, stage IIB is defined. Stage IA doesn't include bloody stool. Proven NEC with absent bowel sounds is divided into two stages. In stage IIA, patients are mildly ill; in stage IIB, they are moderately sick. Metabolic acidosis, thrombocytopenia, ileus, and pneumatosis intestinalis are concomitant with stage IIB. In advanced NEC (stage III), the clinic is severe. In stage IIIA, the bowel is intact. Stage IIIB defines bowel perforation[6].

The data obtained from the electronic files in the hospital's computer system included prenatal (chorioamnionitis, preeclampsia), natal (low birth weight, gestational age, congenital gastrointestinal/non-gastrointestinal malformations, 5th APGAR score, PDA (patent ductus arteriosus), and postnatal risk factors (administration of vasopressor therapy, single or twin delivery, use of mechanical ventilation, the requirement of erythrocyte, fresh frozen plasma transfusions, mortality before). In addition, hematocrit (%) and thrombocyte (/mm^3) values were recorded.

Treatment included conservative medical treatment or surgery for special conditions, including pneumoperitoneum, persistent blood in the stool, and worsening clinical and abdominal findings. Withholding of enteral feedings, gastric decompression, broad-spectrum antibiotics, and parenteral nutrition are the mainstays of treatment. All of our patients received this treatment protocol. In addition, surgical NEC defines definitive intestinal necrosis at surgery or autopsy[7].

The software IBM SPSS V23 was used to analyze the data. We defined parametric data by the mean and standard deviation (S.D.) and nonparametric data by the median and interquartile range. Percentage (%) expressed the enumeration data. Parametric numeric data were calculated using the Independent sample's t-test, and two-sample comparisons of nonparametric data were performed using the Mann-Whitney test. Pearson chi-square, Yates correction, and Fisher's exact test were also used to evaluate the categorical data. P values <0.05 were defined as statistically significant.

Results

Of 1669 preterm neonates who were hospitalized in the Neonatal Intensive Care Unit of Duzce University over 12 years (2009-2021), 27(1.6%) met the criteria ≥IIA of Modified Bell's NEC criteria. In addition, four had stage III NEC, and three underwent surgery.

Necrotizing enterocolitis periods (days) (mean±SD) were similar between different gestational ages: 9±5.03 days for <28 weeks, 13.44±8.41 days for 28-34 weeks, and 15±12.62 days for 34-37 weeks (p=0.614).

We selected 32 controls randomly. We compared the two groups' variables: birth weight, gestational age, maternal chorioamnionitis, preeclampsia, fifth-second APGAR (appearance, pulse, grimace, activity respiration) score, gastrointestinal abnormalities, transfusions, breastfeeding, use of vasopressors, patent ductus arteriosus, and cumulative mortality rates. The mean birth weight±SD of the NEC group was 1.37±0.49 kg vs. 1.83±0.65 kg of the controls (p=0.009). The mean gestational age at birth±SD of the neonates with NEC was 29.95±3.81 weeks vs. 31.88±2.92 weeks of the control group (p=0.073). The distribution of neonates into groups according to the gestational periods (<28 weeks, 28-34 weeks, 34-37 weeks) did not differ between the NEC and non-NEC groups. In addition, the rates of maternal chorioamnionitis and multiple gestations were similar between these groups (Tables 1-2).

Table 1. Demographic characteristics of the study groups

	NEC	Non-NEC	Total	p
Gestational age, n (%)				0.873
< 28 weeks	4 (20)	5 (15.2)	9 (17)	
28-34 weeks	12 (60)	20 (60.6)	32 (60.4)	
34-37 weeks	4 (20)	8 (24.2)	12 (22.6)	
Gestational age (month)	29.95±3.81	31.88±2.92	31.13±3.4	0.073
Birth weight, n (%)				0.374
Small for gestational age	3 (14.3)	2 (6.3)	5 (9.4)	
Normal	18 (85.7)	30 (93.8)	48 (90.6)	
Birth weight (gr) Mean±SD	1367.25±493.62	1831.71±651.62	1649.57±632.3	**0.009**
Gestation, n (%)				0.073
Single	18 (78.3)	30 (96.8)	48 (88.9)	
Multiple	5 (21.7)	1 (3.2)	6 (11.1)	
Chorioamnionitis, n (%)				
Present	1 (4.8)	0 (0)	1 (1.9)	---
Absent	20 (95.2)	31 (100)	51 (98.1)	
Preeclampsia, n (%)				0.491
Present	3 (14.3)	8 (25.8)	11 (21.2)	
Absent	18 (85.7)	23 (74.2)	41 (78.8)	
5^{th} APGAR <5, n (%)				---
Present	2 (9.5)	0 (0)	2 (3.8)	
Absent	19 (90.5)	31 (100)	50 (96.2)	

APGAR: Appearance, pulse, grimace, activity respiration.

Table 2. The characteristics of the included preterm infants

	NEC n (%)	Non-NEC n (%)	Total n (%)	p
GIS malformation				---
Yes	2 (9.1)	0 (0)	2 (3.8)	
No	20 (90.9)	31 (100)	51 (96.2)	
Congenital/genetic non-GIS disease				
Yes	3 (11.5)	0 (0)	3 (5)	
No	23 (88.5)	34 (100)	57 (95)	
Patent ductus arteriozus				0.078
Yes	5 (19.2)	1 (3)	6 (10.2)	
No	21 (80.8)	32 (97)	53 (89.8)	
Poor circulation				**0.004**
Yes	6 (24)	0 (0)	6 (10,2)	
No	19 (76)	34 (100)	53 (89,8)	
Asphyxia/Hypoxia				0.057
Yes	10 (40)	5 (14,7)	15 (25,4)	
No	15 (60)	29 (85.3)	44 (74.6)	
Resuscitation				1.000
Yes	7 (28)	9 (26.5)	16 (27.1)	
No	18 (72)	25 (73.5)	43 (72.9)	
Pulmonary hypertension				
Yes	2 (9.1)	0 (0)	2 (3.6)	
No	20 (90.9)	34 (100)	54 (96.4)	---

Table 3. Comparison of the groups' clinical features

	NEC	Non-NEC	Total	p
Antibiotic administiration >5 days n(%)				
No	4 (16)	32 (97)	36 (62.1)	**<0.001**
Yes	21 (84)	1 (3)	22 (37.9)	
Use of mechanical ventilation n(%)				
Present	16 (59.3)	15 (44.1)	31 (50.8)	0.359
Absent	11 (40.7)	19 (55.9)	30 (49.2)	
Use of vasopressor n(%)				0.008
Present	8 (29.6)	1 (2.9)	9 (14.8)	
Absent	19 (70.4)	33 (97.1)	52 (85.2)	
Hematocrit (%) Mean±SD	39.16±11.04	44.7±11.78	42.55±11.7	0.121
Thrombocyte (/x$10^3$$mm^3$)				
Median (min.-max.)	98 (9 - 2253)	222 (17 - 345)	192 (9 - 2253)	0.012
Red blood cell transfusion 48 hours before NEC, n (%)				1
Present	8 (30.8)	10 (30.3)	18 (30.5)	
Absent	18 (69.2)	23 (69.7)	41 (69.5)	
Use of Fresh frozen plasma, n (%)				0.284
Present	5 (19.2)	3 (9.1)	8 (13.6)	
Absent	21 (80.8)	30 (90.9)	51 (86.4)	
Breastfeeding less than ten days, n (%)				---
Present	9 (39.1)	0 (0)	9 (15.8)	
Absent	14 (60.9)	34 (100)	48 (84.2)	
Mortality before discharge, n (%)				0.037
Present	6 (22.2)	1 (2.9)	7 (11.5)	
Absent	21 (77.8)	33 (97.1)	54 (88.5)	

GIS: Gastrointestinal system; NEC: Necrotizing enterocolitis.

Poor circulation was more frequent in the NEC group (n=6) (24%) than in the non-NEC group (0%) (p=0.004). However, the asphyxia, hypoxia, resuscitation, and pulmonary hypertension history rates were similar between the NEC and non-NEC groups (Table 2).

Comparing the neonates in the NEC group with those in the non-NEC group, the rates of using vasopressor (29.6% vs. 2.9%, respectively) (p=0.008) and cumulative mortality rates (22.2% vs. 2.9%, respectively) (p=0.037) were significantly higher in the NEC group. The rates of having patent ductus arteriosus, using mechanical ventilation, and red blood cell and fresh frozen plasma transfusion were similar. Prolonged use of antibiotics (more than five days) before NEC was significantly associated with NEC (NEC group: 84% vs. non-NEC group: 3%)(p<0.001). In addition, the thrombocyte median (minimum-maximum) level at diagnosis was significantly lower in the NEC group. However, these groups' mean hematocrit levels were similar (Table 3).

Discussion

A recently published meta-analysis, including 28 case-control and 10 cohort studies, confirmed that maternal gestational diabetes mellitus, preeclampsia, prematurity, small for gestational age, sepsis, patent ductus arteriosus, congenital heart disease, mechanical ventilation, and use of antibiotics and vasopressors were risk factors for NEC[5].

Necrotizing enterocolitis of prematurity is a fatal disease. Various risk factors have been identified: prenatal factors (genetics, chorioamnionitis, intestinal immaturity), perinatal factors (low gestational age, low birth weight, abnormal colonization of the intestinal microbiota), and others (environmental stress, mechanical ventilation, central catheters, pharmacological interventions, or antibiotic therapy), a persistent ductus arteriosus (PDA) with or without indomethacin treatment. Also, reduction in placental flow, anemia (Hb≤8 g/dl, but not red cell transfusion), or the prolonged use of antibiotics have been associated with NEC[6].

Despite these defined risk factors, different studies had different conclusions about NEC risk factors[6-16]. Zhang et al.[9] concluded that maternal placenta previa, neonatal infections, septicemia, and use of intravenous aminophylline were significant risk factors for NEC in very

low birth weight infants. However, previously reported risk factors such as maternal hypertension, feeding type, Apgar scores, resuscitation, asphyxia, mechanical ventilation, blood transfusions, PDA, and congenital heart diseases didn't differ between the NEC and non-NEC groups. Wang et al.[10] found that in neonates with NEC and sepsis, the birth weight and gestational age were lower, and anemia, prolonged rupture of membranes (PROM) (≥18 h), pregnancy-induced hypertension, late-onset sepsis (LOS), red blood cell transfusion, and hypoalbuminemia rates were higher than in neonates with sepsis and without NEC.

We found that prolonged use of antibiotics (more than five days) was significantly associated with NEC. Consistent with one of the previous studies, Raba et al.[11] found that prolonged exposure to initial antibiotics for more than five days increased NEC risk by 3.6-fold. Meropenem and gentamicin significantly increased the NEC risk, unlike other antibiotics. In this study, patent ductus arteriosus and its treatment, mechanical ventilation, surfactant therapy, umbilical catheter, and type of feeding didn't differ significantly in NEC cases. Berkhout et al.[12] reported that formulas and prolonged parenteral feeding increased, and antibiotic administration within 24 hours of life decreased the NEC risk. Kordasz et al.[13] demonstrated that low Apgar scores, low hemoglobin, high lactate levels, and congenital heart disease or PDA were associated with severe NEC and mortality in NEC. They also showed that PDA and congenital heart disease tripled the extreme NEC risk. Adult plasma is more viscous than neonates; multiple transfusions with plasma increase neonates' blood viscosity and may impair circulation and cause NEC[14].

Our results agree with some of these findings that birth weight is significantly lower in the NEC group than in the non-NEC group with statistically similar gestational age. The ratios of the preterms with SGA and average weight were statistically identical in these groups. However, we couldn't show the association between NEC and multiple gestations, chorioamnionitis, preeclampsia, Apgar scores, patent ductus arteriosus, and mechanical ventilation. Our small cohort didn't find a significant decrease in pre-NEC hematocrit. Breastfeeding was less than ten days in 9 preterm neonates with NEC and none in the non-NEC group. Pre-NEC plasma transfusion rates were similar between the NEC and non-NEC groups.

Several risk factors, such as transfusion of red cell suspension, hematocrit >49.65%, mean corpuscular volume >114.35 fL, and mean platelet volume >10.95 fL, were reported in one study. On the other hand, the use of pulmonary surfactant, the use of probiotics, and the platelet distribution width >11.8 fL reduced the NEC risk ($p<0.05$)[15].

In contrast to this study, we found that the transfusion of red cell suspension rates were similar between the NEC and non-NEC groups. In addition, the hematocrit of the NEC group (39.16±11.04%) was statistically identical to that of the non-NEC group (44.7±11.78%).

Another notable finding in our study was the significantly lower thrombocyte levels in the NEC group compared with the non-NEC group. The decline of thrombocytes in the early course of NEC is associated with necrotic bowel and worsening disease[16].

Cox et al.[17] pointed out that the administration of caffeine, birth weight, and vasopressors lead to NEC development. The role of vasopressors in NEC development is attributed to vasoconstriction in the intestine. Our results support their findings, including the effect of vasopressors and birth weight in increasing NEC incidence. We also found increased rates of poor circulation before NEC.

Lin et al.[18] reported that of 149 preterm infants, 70.5% were fed by formula before NEC occurred. Prematurity-associated morbidities were significantly higher in VLBW infants. Furthermore, 12.8% of all NEC infants died at discharge. In another study, overall mortality was 23.5% in NEC (Bell stage 2a+) and 34.5% (30.1%-39.2%) for neonates operated for NEC[19].

In our study, preterm neonates with NEC had cumulative mortality rates of 22.2%, which was more significant than the infants without NEC (2.9%) ($p=0.037$).

Conclusions

Mortality rates remain high (22%), with an incidence of 1.6% among preterm infants. Vasopressors and low birth weight are significant risk factors for NEC. In addition, thrombocytopenia, as an alerting sign, is frequent in preterms with NEC.

Ethics Committee Approval: The Duzce University School of Medicine Ethics Committee approved the study (approval number 2022/85). The study was performed under the principles of the Helsinki Declaration.

Peer-review: Externally peer-reviewed.

Authorship Contributions: Concept: H.M.Ç., K.K.; Supervision: H.M.Ç., K.K.; Fundings: None; Materials: H.M.Ç., K.K.; Data collection or Processing: H.M.Ç.; Analysis or Interpretation: H.M.Ç.; Literature Search: H.M.Ç.; Writing and Critical review: H.M.Ç., K.K.

Use of AI for Writing Assistance: Not declared.

Conflict of Interest: None declared.

Financial Disclosure: The authors declared that this study received no financial support.

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