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ORIGINAL ARTICLE

Low Basophil Count and Red Cell Distribution Width at Birth May Predict the Development of Neonatal Necrotizing Enterocolitis: A Matched Control Study

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Objective: The pathophysiology of necrotizing enterocolitis (NEC) is incompletely understood. There were some reports that the pathogenesis of NEC involves intrauterine process and infants with fulminant NEC had low lymphocyte count. Thus, we investigated complete blood count (CBC) parameters of infants at birth and their mothers near delivery.

Methods: We retrospectively reviewed the medical records of NEC patients and controls. The CBC parameters were compared between infants with NEC (modified Bell's criteria stage ≥ Ia, n = 82) and controls matched for gestational age, birth weight, gender, and race (n = 169). The blood test findings were obtained from infants within the first 2 hours of life and from mothers as the latest one before delivery.

Results: Statistically different findings at birth were found in NEC infants; red cell distribution width (RDW) and basophil count. In the multiple logistic regression analysis after adjustment for gestational age, birth weight, and gender, several infantile independent risk factors were identified; basophil count < 40/μL (odds ratio [OR], 4.60; 95% confidence interval [CI], 2.18 to 9.73; P < 0.001) and low RDW (OR, 7.15; 95% CI, 2.93 to 17.41; P < 0.001).

Conclusion: We found that NEC was associated with low infantile RDW and basophil count at birth. These findings might support roles of red blood cell and basophil in the pathogenesis of NEC, which might predict development of NEC with neonatal findings at birth.

Keywords: Basophils; Necrotizing enterocolitis; Erythrocyte indices

INTRODUCTION

Necrotizing enterocolitis (NEC) is a leading cause of death among patients in the neonatal intensive care unit. However, the pathogenesis of NEC is not completely understood but is likely multifactorial [1]. No proven marker to predict the development of NEC was found, while small numbers of studies report the association of neonatal anemia [2], and the patients with fulminant NEC were found to have lower lymphocyte count at the time of diagnosis [3].

In our preliminary data, some maternal red blood cell (RBC) and platelet parameters were significantly associated with proven NEC compared with suspected NEC group. Thus, the authors investigated the association between the development of NEC and the complete blood count (CBC) parameters of the neonates and their mother using gender, gestational age, birth weight, and race matched non-NEC controls to find factors to predict the development of NEC.

MATERIALS AND METHODS

1. Patients and controls

We retrospectively reviewed medical records of NEC patients and non-NEC controls matched for gender, gestational age, birth weight, and race who admitted in neonatal intensive care unit of the Cheonan Hospital of Soonchunhyang University from June 2006 to June 2012. The CBC parameters of NEC patients and their mothers were compared with the matched controls.

A total of 82 NEC (stage ≥ Ia) patients including the extremely

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premature infants were enrolled. The patients were diagnosed by clinical symptoms and signs including the systemic and gastrointestinal symptoms, and the laboratory and radiologic findings according to the modified Bell's criteria.

All cases were matched with 2 control subjects. The 164 control subjects were matched by gestational age (±1 week), birth weight (±80 g), gender, and race. When several infants were close matches, the 2 infants with closest gestational age were chosen. Also infants were matched according to the year of birth to avoid any bias as a result of changes in clinical practice. Once identified from the database, the medical records were reviewed for completeness and to ensure that control subjects did not have any feeding intolerance (feeds withheld for more than 1 day). This study was approved by the ethics review committee of the Medical Research Institute, Soonchunhyang University Medical Center, Cheonan (SCHCM 2012-03-02-01).

2. Collection of blood samples

The data of blood tests within the first 2 hours of life were collected from the neonates by venipuncture or by arterial puncture. The latest blood test data within two days before the delivery were collected from their mothers. The white blood cell (WBC) parameters included total WBC, neutrophil, lymphocyte, and basophil count. The RBC parameters included RBC count, hemoglobin, mean corpuscular volume, and red cell distribution width (RDW). The platelet parameters included the platelet count, platelet distribution width, mean platelet volume (MPV), and the platelet mass (calculated from MPV×platelet count). The variables found to be significant in the univariate analysis were categorized for the multiple logistic regression analysis according to the recent report of CBC reference ranges of premature infants [3-6] (Table 1).

3. Statistical analysis

The data were compared using the chi-square test or Fisher's exact test for the categorical variables and the Student t-test for continuous variables. For the variables that were significantly different between two groups ($P<0.05$), the multiple logistic regression analysis was performed to identify the independent risk factors for the development of NEC and the proven NEC. All analyses were conducted using the PASW SPSS ver. 18.0 (SPSS Inc., Chicago, IL, USA) and $P<0.05$ was considered to be significant.

Table 1. Multiple logistic regression analysis of infantile and maternal parameters between necrotizing enterocolitis group and matched controls after adjustment for gestational age, birth weight, gender, and race[a]

Infantile findings[b]	Odds ratio (95% confidence interval)	P-value
Lymphocyte count (/μL)		
≥4,000	Reference	
<4,000	2.67 (0.97-7.30)	0.057[c]
Basophil count (/μL)		
≥40	Reference	
<40	4.60 (2.18-9.73)	<0.001[c]
Red blood cell distribution width (%)		
Normal	Reference	
Low	7.15 (2.93-17.43)	<0.001[c]

[a]The laboratory parameters were collected from the infants at birth and their mothers of the latest data before the delivery dates. [b]All of infantile complete blood count parameters were dichotomized according to the recent reports of reference ranges for the premature infants considering their gestational age. However, these two parameters were analyzed only for continuous variables because no known reference range for premature infants exists. [c]$P<0.05$.

RESULTS

1. Baseline characteristics

In the clinical characteristics between NEC group (n=82, male:female=42:40) and matched controls (n=164, male:female=84:80) were compared. The gestational age and birth weight were not different between NEC group (32.5±4.2 weeks and 1,814.9±825.7 g) and controls (32.6±4.3 weeks and 1,952.3±1,325.7 g). In addition, there was no difference between two groups in maternal age and the incidence of respiratory distress syndrome, patent ductus arteriosus, sepsis, pneumothorax, chronic lung disease, intraventricular hemorrhage (≥grade 2). However, significantly higher incidence of disseminated intravascular coagulopathy (26.8% vs. 12.8%, respectively), acute renal injury (9.8% vs. 3.0%, respectively), and mortality (19.5% vs. 3.0%, respectively) were found in NEC group (Table 2).

2. Univariate and multivariate comparison of between complete blood count parameters from infants with NEC and their mothers, and matched controls

When the maternal laboratory findings were compared between the NEC group and the matched controls, no significant difference was found. However, statistically lower infantile lymphocyte count (4,285.3±2,262.2 [/μL] vs. 4,791.2±2,193.9 [/μL], $P=0.044$), basophil count (16.5±43.0 [/μL] vs. 60.6±131.4 [/μL], $P<0.001$), and RDW (16.3%±1.7% vs. 16.8%±1.6%, $P=0.034$) at birth were found in the NEC group (Table 3).

Table 2. Clinical characteristics of necrotizing enterocolitis (NEC) groups

Characteristic	Matched controls[a] (n=164)	NEC[a] (n=82)	P-value[b]
Male:female	84:80	42:40	
Gestational age (wk)	32.6±4.3	32.5±4.2	0.817
Birth weight (g)	1,952.3±1,325.7	1,814.9±825.7	0.389
Maternal age (yr)	32.5±16.2	31.9±33.5	0.879
Respiratory distress syndrome	83 (50.6)	47 (57.3)	0.191
Persistent ductus arteriosus	37 (22.6)	27 (32.9)	0.063
Sepsis	32 (19.5)	14 (17.1)	0.205
Disseminated intravascular coagulopathy	21 (12.8)	22 (26.8)	0.004
Acute renal failure	5 (3.0)	8 (9.8)	0.021
Pneumothorax	18 (11.0)	13 (15.9)	0.220
Chronic lung disease	13 (7.9)	6 (7.3)	0.946
Intraventricular hemorrhage ≥ grade 2	25 (15.2)	7 (8.5)	0.178
Death	5 (3.0)	16 (19.5)	<0.001

Values are presented as mean±SD or number (%).
[a]NEC groups included both of suspected and proven NEC of the modified Bell's criteria. [b]Gestational age, birth weight, and maternal age were compared using student t-test and other parameters were analyzed by chi-square test.

These three variables were analyzed by multiple logistic regression analysis after adjustment for the gestational age, birth weight, and gender. The infantile basophil count at birth less than 40/μL (OR, 4.60; 95% CI, 2.18 to 9.73; P<0.001) and low RDW for the gestational age (OR, 7.15; 95% CI, 2.93 to 17.43; P<0.001) were found to be independent risk factors for the development of NEC (Table 1).

DISCUSSION

We showed the lower lymphocyte counts of NEC patients at birth even though it was not significant in multivariate analysis. Interestingly, there was a report that the lower lymphocyte count was significantly related with the development of fulminant cases of NEC [3]. It was explained with the possible infectious involvement in the pathogenesis of NEC. In addition, several evidences implicate the lymphocyte may have an important role in the pathogenesis of NEC [7].

Moreover, we found that infantile basophil count less than 40/μL at birth (reference range for neonates, 30 to 110/μL [8]) increased the risk of NEC by 4.5 times. It was an unexpected but an interesting finding as the function of basophil is unclear yet. Basophil has been known to play a central role for the allergic and parasitic diseases. However, numbers of recent works suggest the basophil is a key factor in the acquired and innate immune responses involving the expression of Toll-like receptors (TLRs) and the immunoregulatory roles via not only the release of cytokines and chemokines, but also its antigen presenting properties [9-11].

Table 3. Univariate comparison between complete blood count parameters from infants and mothers with NEC and controls matched for gestational age, birth weight, gender, and race

Variable	NEC (n=82)	Matched controls (n=164)	P-value
Infantile findings			
WBC count (×10³/μL)	11.6±5.8	12.3±5.8	0.152
Neutrophil count (/μL)	5,251.7±4,199.7	5,836.2±4,348.8	0.312
Lymphocyte count (/μL)	4,285.3±2,262.2	4,791.2±2,193.9	0.044
Basophil count (/μL)	16.5±43.0	60.6±131.4	<0.001
RBC count (×10⁶/μL)	4.4±0.6	4.3±0.5	0.454
Hemoglobin (g/dL)	16.4±2.2	16.2±2.0	0.525
MCV (fL)	113.2±16.3	111.6±7.9	0.417
RDW (%)	16.3±1.7	16.8±1.6	0.034
Platelet count (×10³/μL)	253.6±103.2	243.1±75.4	0.358
PDW (fL)	11.4±1.7	11.3±1.3	0.870
MPV (fL)	10.3±0.8	10.3±0.7	0.802
Platelet mass[a]	2,559.7±1061.6	2,538.0±692.6	0.847
C-reactive protein (mg/dL)	0.9±2.3	1.8±6.7	0.262
Maternal findings			
WBC count (×10³/μL)	12.3±4.6	11.6±6.1	0.320
Neutrophil count (×10³/μL)	9,915.5±4,537.3	10,065.7±4,059.7	0.874
Lymphocyte count (×10³/μL)	1,764.2±620.0	2,066.6±751.8	0.081
Basophil count (/μL)	13.7±9.9	13.9±8.9	0.912
RBC count (×10⁶/μL)	3.9±0.5	3.9±0.4	0.981
Hemoglobin (g/dL)	11.8±1.6	11.8±1.5	0.975
MCV (fL)	88.8±9.9	89.8±7.6	0.358
RDW (%)	13.8±2.0	13.8±1.6	0.993
Platelet count (×10³/μL)	216.3±60.2	220.4±58.0	0.605
PDW (fL)	11.6±2.0	12.0±2.1	0.245
MPV (fL)	10.1±1.0	10.3±1.0	0.168
Platelet mass	2,174.1±557.0	2,247.2±528.3	0.316

Values are presented as mean±SD. The laboratory parameters were collected either from infants of the first day of births and from their mothers of the latest data before the delivery dates. Significant findings are indicated in bold. P<0.05 was considered statistically significant.
NEC, necrotizing enterocolitis; WBC, white blood cell; RBC, red blood cell; MCV, mean corpuscular volume; RDW, red blood cell distribution width; PDW, platelet distribution width; MPV, mean platelet volume.
[a]Platelet mass is calculated from MPV× platelet count (×10³/μL).

As the abnormality in the innate immune response to pathogenic bacteria is considered as one of important pathogenesis, there are some possibilities for the basophil to be involved in this mechanism. In several recent reports, the basophil was issued as an important regulator in the pathogenesis of various non-allergic diseases. For examples, infiltration of basophils in the gastric mucosa has been reported at the sites of *Helicobacter pylori* infection [12] and basophil has been issued as a key factor for the chronic inflammatory damage of kidney diseases [9]. It is interesting that a recent report suggested that the commensal bacteria derived signals regulated basophil hematopoiesis [13], which reminds us the reaction

between commensal and pathogenic bacteria involving TLR pathways seems to be essential in the pathogenesis of NEC [1]. It is necessary to investigate the cytokines associated with basophil function (e.g., interleukin 7, interleukin 13) to help the understanding about the roles of basophil in the pathogenesis of NEC in the future.

Lower infantile RDW was found to be associated with the development of NEC as well. The infants with low RDW at birth had higher risk of NEC by 7.4 times. The anemia of premature infants was reported to compromise the mesenteric blood flow causing intestinal hypoxia and mucosal injury and may increase the risk of developing NEC [2]. However, there was no report on the association between low RDW at birth and the development of NEC. It is not certain why other RBC indices did not show association with development of NEC and what would be its role in the pathogenesis of NEC.

To our knowledge, this is the first report that the neonatal NEC is associated with CBC variables of infants at birth such as low basophil count, RDW, and lymphocyte count. However, there are several limitations in this study. First of all, the small number of study group is one of the limitations. But, it was difficult to enroll larger numbers of patients because the neonatal NEC is a disease entity with low incidence. Secondly, the causes of low RDW or basophil count of neonates were not investigated, which warrant the further study on the roles of RBC and basophil in the pathogenesis of NEC. Thirdly, despite RDW was found to be a risk factor for development of NEC, it does not seem to be clinically significant because of small numeric differences between NEC and control groups (16.8% vs. 16.3%, respectively).

In summary, we found that the low RDW and basophil count of neonates at birth was the independent risk factors for the development of NEC. This finding might support the role of RBC and basophil in the pathogenesis of neonatal NEC, which might help the prediction of the NEC with neonatal CBC findings at birth.

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