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Original article

P-selectin in preterm infants suffering necrotizing enterocolitis

***Background**: Platelet selectin (P-selectin), an adhesion molecule expressed by activated endothelial cells, mediates the early phases of leukocyte adherence to the endothelium. Expression of P-selectin has been shown to be crucial to neutrophil recruitment in many human inflammatory processes as well as in animal models of intestinal ischemia-reperfusion, intestinal transplantation, and sepsis, but its role in NEC is unknown. **Objective**: To study P-selectin, a possible cause of NEC, in the blood of preterm infants. **Study design**: Twenty-four consecutive preterms, clinically suspected or proven to have NEC, were enrolled in this pilot study. Their weight ranged from 1 to 2.3 Kg (mean ±SD: 1.7±0.5 Kg), age ranged from 2 to 21 days (mean ±SD: 12±3.5 days) and their gestational age (GA) ranged from 29 to 33 weeks (mean ±SD: 31±3 weeks). In addition, 12 age- and weight-matched apparently healthy preterm infants served as a control group. Written consents were obtained from the parents of infants included in the study. All neonates were subjected to perinatal history, clinical examination, routine investigations (CBC, plain X-ray and abdominal ultrasonography (US), arterial blood gases and serum bicarbonate, serum sodium, CRP and blood culture), and measurement of blood P-selectin by direct immunofluorescent staining. **Results**: Infants with NEC clinically presented with significant PROM, gastric residual, abdominal distensions, hypoperfusion, hematochezia and evidence of NEC in abdominal X-ray and/or US, compared to control infants. Significant abnormal laboratory investigations in NEC cases included high CRP, hyponatremia, bandemia, thrombocytopenia, metabolic acidosis, and blood culture-proven neonatal sepsis. Abnormal blood P-selectin (>20 units) was detected in 21 (87.5%) infants with NEC, with a mean level of 51±12.4 units that was significantly higher than that of control infants, P<0.001. A strong significant negative correlation was observed between blood P-selectin and each of GA, body weight, platelet count, arterial blood pH and bicarbonate, while it was a significant positive correlation with each of CRP and band cell count. **Conclusion**: P-selectin may have a role in the pathogenesis of NEC in preterm infants and may be used as a diagnostic tool.*

Key words: Prematurity -abdominal distension-hematochezia.

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INTRODUCTION

NEC, the most common gastrointestinal (GI) medical and/or surgical emergency occurring in neonates, is more common in premature than in term neonates[1]. Clinical signs and symptoms range from mild feeding intolerance and abdominal distension to catastrophic disease with bowel perforation, peritonitis and cardiovascular collapse[2]. When conservative medical management fails to halt injury, surgical intervention is often needed[3].

Although many aspects of the pathogenesis of NEC are still unclear evidence suggests that activation of inflammatory cascade with recruitment of leukocytes and production of soluble mediators plays a major role in promoting tissue injury of the intestine and NEC .

Proinflammatory mediators include P-selectin (platelet activating factor-PAF), tumor necrosis factor (TNF), and interleukin (IL) 2, IL-6, IL-10, IL-12 and IL-18[4].

PAF (P-selectin) is responsible for the early stages of leukocyte-endothelial interaction. Within minutes after exposure to appropriate inflammatory stimuli, P-selectin is mobilized and exposed on the cell surface where its interaction with P-selectin glycoprotein ligand-1, a specific ligand expressed

by leukocytes, determines tethering and rolling of neutrophils along the endothelium[5].

Neutrophils are found adhering to endothelial cells expressing P-selectin, supporting the role of P-selectin in promoting neutrophil rolling and adhesion. The degree of neutrophil infiltration in the mucosa is closely related to the intensity of expression of P-selectin. This finding supports a role for neutrophils and P-selectin in actively contributing to the pathogenesis of intestinal damage in NEC[6]. Hence this study aimed to evaluate the level of P-selectin in the blood of preterm infants suffering NEC.

METHODS

This case control was carried out in the Neonatal Intensive Care Unit (NICU) of Zagazig University Children's Hospital, during years 2006 and 2007 on 36 preterm neonates classified into 2 groups :

Patients' group included 24 preterm (14 males and 10 females) clinically suspected or proven to have NEC. Their weight ranged from 1 to 2.3 kg (mean ± SD 1.7±0.5 kg), age ranged from 2 to 21 days (X±SD 12±3.5 days), and their gestational age ranged from 29 to 33 weeks (X±SD 31 ±3 weeks).
Control group included 12 apparently healthy preterms (6 males and 6 females) of mean ages 12.5±4.3 days and mean weight 1.8±0.9 kg.

Ethical approval was obtained from the local research ethics committee and parents of all subjects gave an informed written consent prior to the study.

All preterm neonates were subjected to the following:

1. Perinatal history.
2. Full clinical examination with recording of feeding intolerance, delayed gastric emptying, abdominal signs (distention, ileus, erythema), bleeding (hematochezia, or bleeding diathesis), and cardio-pulmonary signs (e.g., apnea, decreased peripheral perfusion, shock, collapse).
3. Routine investigations: Complete blood counts (CBC), according to Malik et al.[7], serum sodium, arterial blood gases, serum bicarbonate, C-reactive protein (CRP)[9] (positive test above 6mg/L) and blood culture[10] and stool analysis.
4. Abdominal ultrasound and Plain X-ray were done for the patients' group only[8].
5. Measurement of blood P-selectin (CD62p) by direct immunofluorescent staining was measured by flow cytometry, according to Fijnheer et al.[11] using monoclonal Anti-Human CD62p (P-Selectin) Sigma Product No. C5713, Sigma Chemical Co)[11].

A standard curve was created by plotting the absorbance for each standard concentration on the Y axis against concentration provided on the bottles on the X axis .

Statistical analysis[12]

Data were presented as mean ± standard deviation (X±SD) or percentage (%). All statistical comparisons were performed using the student's "t" test or χ^2. Linear correlation and regression were used to test the correlation between P-selectin and other parameters. ROC curve was used to determine the cutoff value to P-selectin. Data were tabulated and statistically analyzed using a computer presentation of data made through SPSS version 19 software package. For all statistical tests done, the threshold of significance was fixed at 5% level (p-value).

RESULTS

The most common significant symptoms and signs of NEC included premature rupture of membranes (PROM), gastric residual, abdominal distension, hypo-perfusion, hematochezia (blood in stools) compared to that in control subjects. Also, abnormal abdominal plain X-ray and/or ultrasound were found in 17 preterms with NEC representing 70.8% of all cases. Meanwhile, weak sucking and apnea were nonsignificant (table 1).

Table 2 presents the laboratory data of 24 preterm infants with NEC versus 12 control infants. A significant rise of CRP and band cell count with significant drop of serum sodium (Na), platelet count, arterial blood pH and serum bicarbonate. Eight cases (33.3%) showed positive blood cultures. Out of 24 cases with NEC 21 cases (87.5%) displayed abnormally high blood P-selectin levels (> 20 units). The mean level of blood P-selectin accounted for 51±12.4 units in NEC cases versus 18±2.1 units in control infants, $P< 0.001$.

Correlation between blood P-selectin and other clinical and laboratory data showed significant negative correlation with gestational age, body weight, platelet count, arterial blood pH and serum bicarbonate. Meanwhile, significant positive correlation was observed with CRP and band cell count (table 3).

Table 1. Clinical characteristics of 24 preterm infants with NEC versus 12 control infants.

Characteristics; n (%)	NEC cases n = 24	Control infants n = 12	OR (95% CI)	P value
PROM	9 (37.5)	0.0	-	<0.001
Weak suckling	21 (87.5)	11 (91.7)	0.64 (0.02-8.53)	0.07
Gastric residual	22 (91.7)	1 (8.3)	121 (7.75-4752.7)	<0.001
Abdominal distension	20 (83.3)	3 (25)	13.33(1.91-113.24)	<0.01
Hypoperfusion	16 (66.7)	0.0	-	<0.001
Apnea	9 (37.5)	4 (33.3)	1.2 (0.23-6.58)	0.8
Blood in stools	14 (58.3)	0.0		<0.001
Abnormal abdominal X-ray or US	17 (70.8)	-	-	

PROM: Premature rupture of membranes, US: Ultrasonography , NEC: necrotizing enterocolitis
P > 0.05: nonsignificant P< 0.05: Significant P< 0.001: Highly significant

Table 2. Laboratory data of 24 preterm infants with NEC versus 12 control infants.

Characteristic	NEC cases n = 24	Control infants n = 12	*t*	*P* value
CRP (mg/L)	21.3±14.4	8±4.3	4.16	<0.001
Serum Na (mEq/L)	115±20.7	139±8.9	4.85	<0.001
Band cell count %	18±6.3	12.3±5.4	2.82	<0.05
Platelet count ($x10^9$/L)	103±40	185±35	6.31	<0.001
pH	7.1±0.2	7.42±0.13	5.77	<0.001
Serum HCO_3 (mmol/L)	15.2±4.6	18.3±3.1	2.39	<0.05
Abnormal blood P-selectin (>20 unit)	21(87.5%)	0.0		
Blood P-selectin X±SD	51±12.4	18±2.1	12.67	<0.001
Positive blood culture, no (%)#	8(33.3%)	0.0	χ^2=5.14	0.023

NEC: Necrotizing enterocolitis CRP: C-reactive protein
p > 0.05: nonsignificant p < 0.05: significant p< 0.001: highly significant
#: All data are expressed as mean ± SD except positive blood culture

Table (3) Correlation between blood P-selectin and other parameters in 24 preterm infants with NEC

Parameter	"r"	*P*
Gestational age (weeks)	-0.41	< 0.05
Body weight (Kg)	- 0.51	< 0.01
CRP	+ 0.57	< 0.01
Band cell count	+ 0.9	< 0.001
Platelet count	- 0.47	< 0.05
pH and bicarbonate	- 0.57	< 0.01

CRP: C-reactive protein, NEC: Necrotizing enterocolitis, p > 0.05: nonsignificant, p < 0.05: significant, p< 0.001: highly significant

DISCUSSION

Many variables are associated with the development of NEC, but only prematurity has been identified as the main risk factor. Preterm infants account for 70% to 90% of total NEC cases. The more preterm the infant, the higher the risk. By 36 weeks of gestation, there is sharp decrease in the incidence of NEC. The specific vulnerability of the preterm infant's GI tract to NEC is likely related to an immature GI mucosal barrier and immune response, along with impaired circulatory dynamics from hypoxic-ischemic insults. Premature infants may experience vasoconstriction, hypotension and thrombosis leading to decreased GI perfusion[13].

After exposure to appropriate inflammatory stimuli, P-selectin is mobilized and exposed on cell surface, where its interaction with its ligand determine tethering and rolling of neutrophils along the endothelium[l4]. Activated neutrophils release a wide array of proteolytic enzymes which may result in excessive host-tissue damage, more infiltration of neutrophils in the intestine and development of tissue damage in NEC[15].

The most common signs and symptoms of NEC encountered in our series were gastric residual (91.7%), abdominal distention (83.3%), hypoperfusion (66.7%), bloody stools (58.3%) and abnormal abdominal X-ray and/or US (70.8%). Similar results were obtained by Hsueh et al.[16]. However, the delay of enteral feeding in sick preterm neonates may decrease the normal GI functional adaptation, resulting in subsequent feeding intolerance[17].

In our study, serum sodium, arterial blood pH, and serum bicarbonate were found to be significantly lower in preterm infants with NEC than that in control infants, similar to what was reported by Kling and Hutter[18]. Hyponatremia is a

worrisome sign that can suggest the initial stages of developing capillary leak. Meanwhile, metabolic acidosis results from decreased cardiac output, leading to poor perfusion of peripheral tissues and lactic acidosis[19]. Also, the platelet counts were significantly decreased in infants with NEC than in control infants and this was in agreement with Jeng-Jung et al.[20] who stated that P-selectin dependent platelet aggregation and apoptosis may explain the decrease in platelet count[20]. The absence of NEC in the sterile inutero bowel and in stillborn infants strengthens the role of infection in its pathogenesis. In addition, the radiographic hallmark of NEC, pneumatosis intestinalis, signifies intramural gas which is believed to be a result of hydrogen gas produced during the bacterial fermentation of enteral feedings. Thus, functional GI immaturity in the presence of gram-negative organisms may trigger the inflammatory process resulting in tissue injury and ultimately NEC[21]. Strains of *E. coli* not previously recognized as pathogenic are able to translocate across mucosal cell layers and enter the lymphatic system or blood stream[22].

Markers of neonatal infection or sepsis, namely CRP, increased band cell count and positive blood cultures, were significantly abundant in preterm infants with NEC than in control infants. Similar results were reported by other studies[7,9,10].

In this study, abnormal blood P-selectin (> 20 units) was detected in the majority (87.5%) of infants with NEC. Meanwhile, a significant rise of the mean value of P-selectin was detected in NEC cases compared to that in control infants. Stefanotti et al.[6] reported that expression of P-selectin is increased in medium-sized vessels and in the microcirculation in intestinal specimens of neonates with NEC compared with neonatal controls[6]. Moreover, Hsueh et al.[23] reported that patients with NEC show high levels of PAF and decreased plasma PAF-acetylhydrolase, the enzyme degrading PAF[23].

Furthermore, a significant positive correlation was observed between blood P-selectin and each of CRP and band cell counts. Similar results were obtained by Kingsmore et al.[24] who reported significant alternation in levels of eight serum proteins specifically P-selectin and CRP[24]. On the other hand there was a significant negative correlation between blood P-selectin and each of gestational age, body weight, blood PH and serum bicarbonate. These results are in agreement with Kingsmore et al.[24]. Also, a significant negative correlation was found between blood P-selectin and platelet count, similar to what was reported by Jeng-Jung et al.[20] who explained the decrease in platelet count by P-selectin dependent platelet aggregation and apoptosis[20].

In conclusion, blood p-selectin was detected in the majority of preterm infants with NEC and was significantly higher compared to controls. These results may explain the role of P-selectin in pathogenesis of NEC. So we recommend further studies to explain the role of other proinflammatory mediators in the pathogenesis of NEC.

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