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Original Research Article

Role of Fecal Calprotectin in Detection of Gastrointestinal Injury in Preterm Infants

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Abstract: Necrotizing enterocolitis (NEC) is a devastating bowel disease, preterm face. This study aimed to explore the role of fecal calprotectin (FCP) as a noninvasive marker of gastrointestinal (GI) injury in preterm infants to detect its usefulness in diagnosis and prediction of NEC severity. On 38 preterm infants a prospective cohort study was carried out. Eighteen preterm infants showed feeding intolerance (FI) and 20 showed feeding tolerances (controls). The preterm infants were recruited from the neonatal intensive care unit, Benha University Hospital, from June 2014 to Dec 2015. FCP was estimated by ELISA in Baseline and follow-up samples. Hematologic parameters were also estimated. FCP showed non-significant baseline difference but was significantly increased at the 2nd sample in FI compared to controls. Within the FI group, highly significantly increased FCP was found in follow-up samples (2nd and 3rd) compared to the 1st. FCP (2nd and 3rd) significantly elevated with increased severity of enteropathy grades. FCP (3rd) was highly significantly increased in died compared to survived infants. Significant positive correlations between FCP (2nd and 3rd) with both Bell's staging and CRP were found. Significant negative correlations between FCP (2nd) and platelets, pH, pCO_2 and HCO_3^- but significant negative correlations between FCP (3rd) and hemoglobin, hematocrit, platelets, pH, HCO_3^- and sodium were found. FCP cutoff levels; 120.5µg/g for NEC occurrence, 128.7µg/g for definite NEC and 215.5µg/g for advanced NEC were determined. To conclude, our study showed increased FCP in preterm with GI inflammation (particularly NEC) compared to controls, with a significant positive correlation with NEC severity. FCP could distinguish NEC from more benign FI. Serial measurements might be a useful non-invasive tool for prediction of NEC severity and prognosis.

Keywords: fecal calprotectin, feeding intolerance, necrotizing enterocolitis, noninvasive tool, ELISA

INTRODUCTION

Necrotizing enterocolitis (NEC) is one of the most common and serious acquired bowel diseases a premature newborn can face [1]. NEC diagnosis currently relies on modified Bell's staging criteria which may overlap with other clinical conditions. This diagnostic uncertainty can result in improper medical management [2].

Identification of a reliable marker, able to predict NEC onset, unambiguously diagnose, predict severity and prognosis, and determine resolution is important. Many serological markers, evaluated for diagnosing NEC, identify systemic inflammation and lack sufficient specificity [3]. A noninvasive sampling is preferred especially in preterm infants to avoid pain and anemia caused by frequent blood sampling [4].

Calprotectin is a 24kDa heterodimer of calcium binding proteins S100A8 and S100A9 [5]. Calprotectin, released by leukocytes at the site of inflammation, is easily measured in feces being stable for one week. Increased fecal calprotectin (FCP) levels are found in several inflammatory conditions, mainly inflammatory bowel diseases [6]. This study aimed to explore the role of FCP as a noninvasive marker of gastrointestinal (GI) injury in preterm infants to detect its usefulness in diagnosis and prediction of NEC severity.

METHODS

This prospective cohort study was carried on 38 preterm infants, recruited from the neonatal intensive care unit (NICU), Benha University Hospital, from June 2014 to Dec 2015. Approval of the study by the Ethical Scientific Committee of the Faculty of Medicine, Benha University was obtained, with informed consents obtained from all parents of included newborns.

Study design

Preterm infants (≤34 weeks) were followed for early signs of GI injury manifested by feeding intolerance (FI). GI congenital anomalies, newborns failed to pass stool, died before sampling or due to other complications were excluded. Infants were divided accordingly into 2 groups; Group I: 18 infants with FI: gastric residual volume >50% of prior feed, abdominal girth increase >1.5cm accompanied with other FI symptoms, emesis >3times/24hours with bilious, blood stain, blood or guaiac-positive stools [7]. Group II: 20 infants achieved *full gavage feedings* with no FI; they achieved appropriate weight gain (~15g/kg/day) without parenteral nutrition [8]. This occurred on receiving 120kcal/kg/day (150ml/kg/day).

All preterm infants were subjected to full history taking and thorough clinical examination stressing on gestational age assessment using expanded Ballard score [9], anthropometric measures, correlation with centiles, vital data, general examination (conscious state, neonatal reflexes, color and perfusion) and systemic examination (especially abdominal assessing intestinal sounds and GI injury signs). GI injury severity was determined by modified Bell's staging criteria for NEC. Plain x-ray abdomen was done for group I.

Sampling and Laboratory investigations:

Blood samples were taken as a part of routine assessment in the NICU; 1ml venous blood on ethylene-diamine tetra-acetic acid for complete blood count (CBC), 1ml venous blood centrifuged to separate serum for C-reactive protein (CRP) and 1ml heparinized capillary blood for arterial blood gases (ABG) and electrolyte measurement. Fecal samples were collected before the start of feeding for baseline FCP (1^{st} sample). Feeding tolerance was assessed daily starting on the first day that feedings were attempted. On FI symptom appearance, a 2^{nd} sample was obtained. FI infants were enrolled in group I. Preterm infants who achieved full gavage feeding with no FI were served as controls (group II). When feeding reached ~120kcal/kg/day (150ml/kg/day) a 2^{nd} sample was obtained from controls. In group I, a 3^{rd} sample (follow up) was collected 3-5 days later to evaluate FCP correlation with the degree of GI injury and relation to prognosis.

FCP measurement by enzyme linked immunosorbent assay (ELISA)

Fecal samples were immediately stored at -20^{o}C. FCP was measured by EDI™ Quantitative Fecal Calprotectin ELISA kit (Epitope Diagnostics, Inc., CA, and USA). A stool specimen (50-100mg) was diluted twice (1:40 then 1:9 with fecal extraction buffer); 50µl clear extract was used for the assay. Optical density was measured at 450nm by TECAN Infinite F50 ELIZA Reader (Singapore). A point-to-point fitting standard curve was generated with result calculation using Magellan Tracker software (Tecan Trading AG, Switzerland).

STATISTICAL ANALYSIS

The data were analyzed using SPSS version 16. Quantitative data were expressed as Mean±SD and qualitative data were expressed as percentages. Student's *t*-test was used to compare mean of quantitative data among two groups, paired t test to compare mean of variables of quantitative data in the same group. ANOVA test (F value) was used to compare mean of quantitative data among more than 2 groups of with post-hoc test (LSD) for multiple comparisons. Z test was used to compare proportion between two groups of qualitative data. Inter-group comparison of categorical data was performed using chi square test (X^2), fisher exact test (FET). Correlation coefficient (r) was used to find relationships between variables. Cutoff values were determined using the receiver operating characteristics (ROC) curve analysis. $p<0.05$ was significant and $p<0.01$ was highly significant**.**

RESULTS

In the current study, no statistically significant differences could be detected between the studied groups as regard characteristic data and feeding criteria [Table 1].

At the 1^{st} (baseline) sample; there was non-significant FCP and other laboratory investigations between the studied groups. However, at the 2^{nd} sample; there were highly significant increases in FCP, leucocytes and CRP (p=0.001 for all) but significant decreases in platelets (p=0.001), pCO_2 (p=0.001) and HCO_3^- (p=0.001) and pH (p=0.48) [Table 2].

In group I, FCP was highly significantly increased in serial stool samples (2^{nd} and 3^{rd}) (154.49±59.27 and 166.36±53.95, respectively) compared to the 1^{st} sample (40.98±19.59) (p=0.001). There was a highly significant progressive increased CRP in serial samples (p=0.008). However, there were highly significant decreases in hemoglobin (p=0.001), hematocrit (p=0.001), platelets (p=0.001) and pCO_2 (p=0.006) in the 2^{nd} and 3^{rd} samples compared to 1^{st}. Also, there was a highly significant decrease in

leucocytes (p=0.001), pH (p=0.002), HCO_3^- (p=0.001) and sodium (p=0.001) in the 3rd sample compared to 1st and 2nd [Table 3].

There were statistical significant differences in FCP at 2ndand 3rd samples within different grades of enteropathy among group I. At the 2nd sample, infants were distributed according to Bell's staging; 22.2% (stage 0), 22.2% (stage Ia), 16.7% (stage Ib), 22.2% (stage IIa) and 16.7% (stage IIb) with significantly increased FCP levels as the severity increased (102±9.91, 137.51±66.8, 148.97±20.26, 165.9±49.87 and 237.4±46.67, respectively, p=0.023). At the 3rd sample, infants were also distributed according to Bell's staging; 50% (stage 0), 16.7% (stage IIb), 22.2% (stage IIIa) and 1.1% (stage IIIb) with significantly increased FCP levels as the severity increased (38.67±18.12, 224.63±66.32, 321.83±109.88 and 406.45±42.78 respectively, p=0.001) [Table 4].

Table 1: Characteristic data and feeding criteria of the studied groups

Variables	Group I (n.=18)	Group II (n.=20)	Test	p
	n.(%) or Mean±SD			
Sex (Male/Female)	10(55.6%)/8(44.4%)	9(45%)/11(55%)	X^2=0.422	0.516
Mode of delivery (CS/NVD)	9(50%)/9(50%)	9(45%)/11(55%)	X^2=0.095	0.758
Gestational age (weeks)	31.56±1.10	31.55±1.64	0.012	0.99
Birth weight (grams)	1426.1±148.0	1462.5±206.6	0.618	0.541
Maternal diseases				
PROM/PE/PE&PROM	2(11.1%)/1(5.6%)/1(5.6%)	3 (15%)/2(10%)/0(0%)	FET=1.56	1.0
Neonatal diseases				
Respiratory distress syndrome	13(72.2%)	11(55%)	Z=0.41	0.341
Early onset sepsis	0(0%)	4(20%)	-	-
Late onset sepsis	12(66.6%)	9(45%)	Z=0.456	0.324
Small for gestational age	3(16.7%)	4(20%)	Z=0.382	0.351
Patent ductus arteriosus	2(11.1%)	3(15%)	Z=0.456	0.324
Intra-ventricular hemorrhage	1(5.6%)	0(0%)	-	-
Lung collapse	1(5.6%)	0(0%)	-	-
Air leak	1(5.6%)	0(0%)	-	-
Feeding criteria				
Start of feeding (days)	4.28±2.02	4.75±2.4	0.651	0.519
Breast feeding/Formula/Mixed	6 (33.3%)/5(27.8%)/7(38.9%)	10 (50%)/6(30%)/4(20%)	FET=1.8	0.427
Number of feeds/day	7.22±2.07	8.40±2.87	1.43	0.16
Initial rate (ml/kg/d)	17.66±8.78	21.47±9.33	1.29	0.205
Rate of increment (ml/kg/d)	13.47±6.49	17.12±5.31	1.9	0.065

CS: caesarian section, NVD: normal vaginal delivery, PROM: premature rupture of membranes, PE: preeclampsia

Table 2: Laboratory parameters of the studied groups (1st and 2nd samples)

Variables	1st sample				2nd sample			
	Group I (n.=18)	Group II (n.=20)	St t test	p	Group I (n.=18)	Group II (n.=20)	St t test	p
	Mean±SD				Mean±SD			
FCP (µg/g)	40.98±19.59	42.31±23.22	0.189	0.851	154.49±59.27	35.90±15.72	8.63	0.001**
Hemoglobin (gm/dl)	15.32±1.02	15.31±1.45	0.004	0.997	13.17±1.93	13.1±1.51	0.119	0.906
Hematocrit (%)	48.47±3.61	47.58±4.60	0.659	0.514	39.91±5.99	39.88±4.57	0.018	0.986
Leucocytes ($x10^9$/L)	11.58±3.63	11.33±3.98	0.204	0.938	12.47±3.43	8.46±1.39	4.82	0.001**
Platelets ($x10^9$/L)	233±32.33	228±56.81	0.348	0.73	164±40.40	272±44.647	7.84	0.001**
CRP (mg/L)	7.39±6.31	11.55±20.16	0.838	0.407	26.06±24.81	4.5±0.51	3.89	0.001**
pH	7.38±0.027	7.37±0.04	0.784	0.438	7.36±0.05	7.39±0.02	2.05	0.048*
pCO_2 (mmHg)	43.49±3.79	44.83±4.55	0.976	0.336	37.51±4.5	41.68±1.28	3.98	0.001**
HCO_3^- (mmol/L)	24.26±1.27	24.61±1.18	0.893	0.378	22.44±1.74	24.39±0.86	4.45	0.001**
Sodium (mmol/L)	140.11±3.32	139.85±3.69	0.228	0.821	139.5±4.64	140.1±2.1	0.522	0.605
Potassium (mmol/L)	4.30±0.55	4.29±0.63	0.023	0.982	4.3±0.61	4.37±0.44	0.42	0.677

FCP: fecal calprotectin, CRP: C-reactive protein, pH: power of hydrogen, pCO_2: partial pressure of carbon dioxide, HCO_3^-: bicarbonate, *: significant, **: high significant

Table 3: Laboratory parameters in serial samples among group I

	Group I (n.=18)				
Variables	**1st sample**	**2nd sample**	**3rd sample**	**ANOVA**	**p**
	(Mean±SD)				
FCP (µg/g)	40.98±19.59	154.49±59.27[a]	166.36±53.95[a]	9.74	0.001**
Hemoglobin (gm/dl)	15.32±1.02	13.17±1.93[a]	11.99±2.66[a]	12.95	0.001**
Hematocrit (%)	48.47±3.61	39.91±5.99[a]	37.33±9.45[a]	13.27	0.001**
Leucocytes (x10^9/L)	11.58±3.63	12.47±3.43	7.68±4.49[a,b]	7.8	0.001**
Platelets (x10^9/L)	233±32.328	164±40.401[a]	137±10.419[a]	9.78	0.001**
CRP (mg/L)	7.39±6.31	26.06±24.8[a]	50.22±63.54[a,b]	5.31	0.008**
pH	7.38±0.027	7.36±0.05	7.28±0.14[a,b]	7.27	0.002**
pCO_2 (mmHg)	43.49±3.79	37.51±4.5[a]	37.98±8.38[a]	5.7	0.006**
HCO_3^-(mmol/L)	24.26±1.27	22.44±1.74	18.98±5.97[a,b]	9.61	0.001**
Sodium (mmol/L)	140.11±3.32	139.5±4.64	132.1±10.4[a,b]	7.53	0.001**
Potassium (mmol/L)	4.3±0.55	4.3±0.61	4.47±0.87	1.1	0.09

FCP: fecal calprotectin, CRP: C-reactive protein, pH: power of hydrogen, pCO_2: partial pressure of carbon dioxide, HCO_3^-: bicarbonate, [a]: significant compared to 1st sample, [b]: significant compared to 2nd sample, **: high significant

Table 4: FCP among group I in different grades of enteropathy (2nd and 3rd samples)

Group I (n.=18)	Bell's staging	n.(%)	FCP (µg/g)	ANOVA	p
2nd sample	0	4(22.2%)	102±9.91	4.13	0.023*
	Ia	4(22.2%)	137.51±66.8		
	Ib	3(16.7%)	148.97±20.26		
	IIa	4(22.2%)	165.9±49.87		
	IIb	3(16.7%)	237.4±46.67		
3rd sample	0	9(50.0%)	38.67±18.12	34.71	<0.001**
	IIb	3(16.7%)	224.63±66.32		
	IIIa	4(22.2%)	321.83±109.88		
	IIIb	2(11.1%)	406.45±42.78		

*: significant, **: high significant

There was a high significant increase in the follow up FCP (3rd sample) in the nine died group I infants (308.23±104.79) compared to the nine survived infants (38.67±18.12) (Student t test=7.60 and p=0.001) [Figure 1].

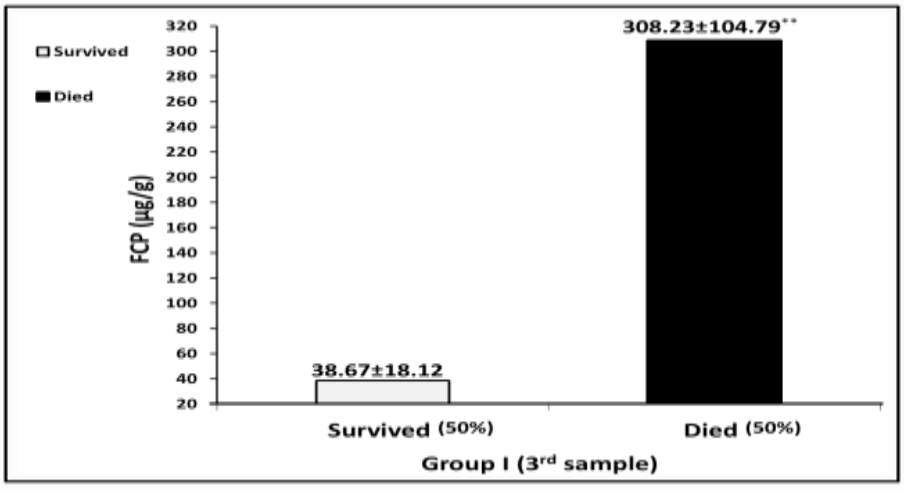

Fig 1: Follow up FCP (3rd sample) as regard survival among group I

Figure 1 legend: A high significant increase was detected in the follow up FCP (3rd sample) in died compared to survived infants among group I (Student t test=7.60 and p=0.001).

Statistically significant positive correlations between FCP (2nd and 3rdsample) with both Bell's

staging and CRP were found among group I. However, there were statistically significant negative correlations between FCP 2nd sample and platelets, pH, pCO_2 and HCO_3^-. Moreover, statistically significant negative correlations between FCP (3rd sample) and hemoglobin, hematocrit, platelets, pH, HCO_3^- and sodium were found [Table 5].

Three cutoff levels of FCP (2nd sample) were determined; 120.5µg/g for occurrence of NEC with 92.9% sensitivity, 100% specificity and area under curve (AUC) 0.929, 128.7µg/g for occurrence of definite NEC (Bell's stage ≥IIa) with 100% sensitivity, 63.6% specificity and AUC 0.844 and 215.5µg/g for occurrence of advanced NEC (Bell's stage ≥IIIa) with 100% sensitivity, 91.7% specificity and AUC 0.972. [Table 6, Figure 2]

Table 5: Correlation between FCP (2nd and 3rd samples), Bell's staging and other laboratory parameters among group I

Group I (n.=18)	FCP (µg/g)			
	2nd sample		3rd sample	
	r	p	r	p
Bell's staging	0.705	0.001**	0.543	0.02*
Hemoglobin (gm/dl)	-0.402	0.098	-0.798	0.001**
Hematocrit (%)	-0.413	0.089	-0.800	0.001**
Leucocytes ($x10^9$/L)	0.099	0.695	-0.208	0.407
Platelets ($x10^9$/L)	-0.562	0.015*	-0.912	0.001**
CRP (mg/L)	0.684	0.001**	0.579	0.009**
pH	-0.48	0.044*	-0.883	0.001**
pCO_2 (mmHg)	-0.704	0.001**	-0.335	0.174
HCO_3^- (mmol/L)	-0.67	0.002**	-0.929	0.001**
Sodium (mmol/L)	-0.459	0.055	-0.875	0.001**
Potassium (mmol/L)	-0.202	0.423	0.337	0.170

FCP: fecal calprotectin, CRP: C-reactive protein, pH: power of hydrogen, pCO_2: partial pressure of carbon dioxide, HCO_3^-: bicarbonate, *: significant, **: high significant

Table 6: Cutoff value of FCP (2nd sample) for NEC according to Bell's stage

Group I (2nd)	Cutoff (µg/g)	Specificity %	Sensitivity %	PPV %	NPV %	Accuracy	AUC	p
Bell's stage ≥Ia†	120.5	100	92.9	100	80.0	94.4	0.929	0.002**
Bell's stage ≥IIa‡	128.7	63.6	100	63.6	100	77.8	0.844	0.028*
Bell's stage ≥IIIa‼	215.5	91.7	100	85.7	100	94.4	0.972	0.001**

†: occurrence of NEC, ‡: occurrence of definite NEC, ‼: occurrence of advanced NEC, *: significant, **: high significant, PPV: positive predictive value, NPV: negative predictive value, AUC: area under curve

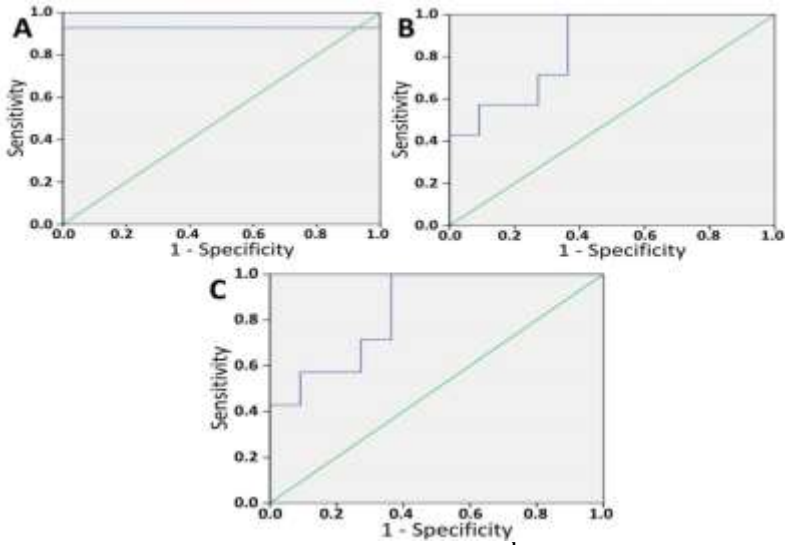

Fig 2: ROC curves of FCP (2nd sample)

Figure 2 legend: ROC curves of FCP (2nd sample); A: for occurrence of NEC (Bell's stage ≥Ia), B: for occurrence of definite NEC (Bell's stage ≥IIa) and C: for occurrence of advanced NEC (Bell's stage ≥IIIa).

DISCUSSION

The present study found no significant differences between baseline (1st sample) laboratory investigations among the studied groups. However, Cui and Li found significantly higher 1st FCP in preterm with FI than those with feeding tolerance [10]. In accordance with our study concerning other laboratory investigations, non-significant differences were previously reported between NEC and non-NEC as regard leucocytes, platelets, hemoglobin, hematocrit and ABG [11], initial CRP level [12] and pH [13].

As regard 2nd sample, FCP was significantly increased in group I compared to group II. In agreement, significantly higher 2nd FCP was found in preterm with FI compared to those with tolerance [10] and in NEC compared to controls [14], and to other diagnoses [4]. Moreover, significant elevated FCP was found at and 48 hours before NEC onset [15] and in suspected NEC infants who finally developed NEC (Bell's stage ≥II) [16, 17]. Our finding could be explained by the ejection of nuclear chromatin and bactericidal proteins in a structure known as neutrophil extracellular traps (NETs) to trap and kill microorganisms in a process termed NETosis [18]. FCP in excised NEC-affected bowel is one of the proteins released by neutrophils in association with NETs [17]. Also, NEC is an inflammatory intestinal disorder characterized by sequestration of neutrophils into the gut wall. Intestinal neutrophil influx followed by activation, results in degranulation and release of calprotectin [19]. In contrast, other studies showed that FCP did not differ between preterms with and without NEC. They explained this by the small sample size [20] or early disease stage of the studied newborns [21].

In our study, in group I (2nd sample), there were high significant increases in leucocytes and CRP but significant decreases in platelets, pH, pCO_2 and HCO_3^- compared to group II. Although significant, all values were within normal for this age group [22]. In agreement with our results, Hällström *et al.;* showed significant lower pH and platelets (platelets still within normal) with non-significant hematocrit, sodium and potassium at NEC onset compared to controls [23]. Moreover, significant increases in leucocytes and neutrophils in NEC were also obtained [24]. In other studies, slightly increased immature to total neutrophils ratio [17] and higher CRP with no significant differences in hemoglobin and hematocrit were also reported [11]. In contrast, previous studies reported non-significant platelet count and CRP level but significant lower leucocytes [19], significantly decreased hemoglobin, [24] pH and ABG levels in NEC versus other diagnoses [11].

In our study, as regard serial fecal samples in group I, there was highly significant increased FCP at 2nd and 3rd samples compared to 1st sample. In accordance, Aydemir *et al.;* and MacQueen *et al.;* reported significantly higher FCP in the follow-up sample of NEC compared to controls in 1st and 2nd samples. In stage III NEC, FCP increased even more in 2nd sample, although not significant. In contrast, in stage II NEC, FCP decreased significantly in 2nd sample [13, 17].

As regard laboratory parameters of serial samples among group I, there were highly significant decreases in hemoglobin, hematocrit, platelets and pCO_2 in the 2nd and 3rd samples compared to the 1st. Also, there was a highly significant decrease in leucocytes, pH, HCO_3^- and sodium in 3rd sample compared to 1st and 2nd samples. Moreover, there was a highly significant progressive increased CRP in serial samples. Hällström et al. showed a drop in hemoglobin, hematocrit, platelets and pH with disease progression [23]. In addition, significantly dropped platelets from the day of diagnosis to the day prior operation [25] and higher CRP in NEC; at diagnosis and 3 days later [13] were also reported.

Our hematologic results could be explained by that: Thrombocytopenia is present in 50-95% of NEC infants. Advanced NEC develops thrombocytopenia 24-72 hours of disease onset that may be primarily due to increased platelet destruction. NEC patients may develop anemia due to multiple pathogenic mechanisms. In mucosal injury, thrombocytopenia, and coagulation disturbances, occult and obvious blood loss are common. Hemolysis by thrombotic microangiopathy can further worsen anemia. Finally, NEC infants may be at increased risk of inflammatory suppression of erythropoiesis. Neutrophilia comprise an appropriate inflammatory response in NEC, however, neutropenia may be associated with adverse outcome in severe NEC [26].

The current study showed increased FCP with the increased disease severity and statistically significant positive correlations between FCP (2nd and 3rd samples) and degree of enteropathy (Bell's staging) among NEC infants. In agreement with the present study, Aydemir *et al.;* reported a significant positive correlation between FCP and NEC severity [27], moreover, Albanna *et al.;* observed that FCP was maximum in Bell's stage IIIb [24].

In contrast to our results, Däbritz *et al.;* found that FCP did not differ with disease severity [15]. Moreover, Zoppelli et al.; observed elevated FCP 12–48 hours before clinical signs in moderate NEC, however, FCP was low in fulminant NEC. They explained their results by the impaired recruitment of granulocytes in the GI lumen of VLBW infants which could make them at risk for aberrant postnatal microbial colonization and

subsequent NEC [12].

We studied the follow-up FCP (3rd sample) as regard survival among patients. Half the patients died and the other half improved. FCP (3rd sample) was highly significantly increased in died cases compared to the survived. Our result agreed with Albanna *et al.;* who reported that 33.3% of their patients improved and 66.7% died with highly significantly increased FCP in died patients [24]. On the other hand, FCP did not significantly differ in fatal compared to medical NEC [25].

As regard the correlations, there was a statistically significant negative correlation between FCP (2nd sample) and platelets, pH, pCO_2 and HCO_3^- and between FCP (3rd sample) and hemoglobin, hematocrit, platelets, pH, HCO_3^- and sodium. However, there was a statistically significant positive correlation between FCP and CRP in 2nd and 3rd samples. Our results agreed with Aydemir *et al.;* who showed a significant positive correlation between FCP and CRP at diagnosis and 3 days later in NEC [13] but no correlation between FCP and CRP in non-NEC suggesting that systemic infection does not affect FCP level in absence of severe abdominal disease [20]. In a previous study, significantly decreased platelets with increasing NEC severity were found [25]. In addition, severe NEC (with bowel perforation) was more likely to exhibit thrombocytopenia, significant increased CRP, and relatively low pH compared to those without perforation [28]. Moreover, it was concluded that hyponatremia, severe acidosis and thrombocytopenia might be indicators of clinical worsening in NEC [29]. All of the above findings together with Maheshwari *et al.;* study on immunologic and hematological abnormalities in NEC may support our findings regarding correlation between FCP and laboratory parameters [26].

To distinguish NEC from more benign FI forms and predict disease progression, different cutoff levels were estimated; 120.5µg/g for occurrence of NEC (92.9% sensitivity, 100% specificity and AUC 0.929), 128.7µg/g for definite NEC (100% sensitivity, 63.6% specificity and AUC 0.844) and 215.5µg/g for advanced NEC (100% sensitivity, 91.7% specificity and AUC 0.972).

Zoppelli *et al.;* detected a FCP cutoff 110µg/g showing 89% sensitivity and 87% specificity to detect moderate NEC 24 hours before clinical symptoms, whereas a cutoff 210µg/g with 89% sensitivity and 84% specificity was reported for definite NEC [12]. Thuijls *et al.;* reported that a cutoff 286.2µg/g with 93% sensitivity and 86% specificity discriminated NEC from suspected NEC with other final diagnoses [19]. Josefsson *et al.;* suggested a cutoff 2000µg/g for identifying severe intestinal inflammation [20].

CONCLUSIONS

Our results showed a substantial difference between FCP levels in preterm infants with features of GI inflammation (particularly NEC) compared to controls, with a significant positive correlation with NEC severity. FCP level could help distinguish NEC from more benign forms of FI. Serial FCP measurements might be a useful non-invasive tool for prediction of NEC severity and prognosis and help planning the length of enteral fasting, total parenteral nutrition and antibiotics.

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