BENHA MEDICAL JOURNAL bmj
Print ISSN 1110-208X.
Online ISSN 2357-0016

Original article

Study the Value of Fecal Calprotectin in Neonate with Congenital Heart Disease and Necrotizing Enterocollitis

Somaia A. Elwan[a] , Abeer E. Karish[a], Maha M. Osman[b], Eman G. Abdelrahman[a]

Abstract:

Background: Neonates with congenital heart disease (CHD) are riskier for suspected necrotizing enterocolitis (NEC) due to reduced cardiac output causing decreased mesenteric blood flow and gut ischemia. Fecal calprotectin levels are elevated in infectious and inflammatory illnesses like NEC. The purpose of this work was to assess the role of fecal calprotectin in the early recognition of suspected NEC in infants with CHD. **Methods:** This case control research has been performed on 80 neonates, both sexes, divided into 3 groups: group 1: CHD whether cyanotic or a cyanotic, group 2: CHD with suspected NEC (abdominal distention, gastric residual, bloody stool), group 3: control healthy group. Radiological and laboratory investigations were done. Fecal calprotectin markers were measured at the time of suspecting NEC and were correlated to gestational age, weight, length, vital signs, other laboratory markers. **Results:** Fecal calprotectin level, C-reactive proteins, prothrombin time, international normalized ratio and serum glutamic-pyruvic transaminase were significantly greater in the CHD and suspected NEC group, there is a significant negative association has been observed among fecal calprotectin and weight (r = -0.449, P-value equal to 0.047) and creatinine (r = -0.454, P = 0.044) in this group. Additionally, fecal calprotectin was a significant predictor of CHD with suspected NEC, controlling for gestational age, sex, weight, and type of feeding. **Conclusions:** Neonates with CHD and suspected NEC had higher level of fecal calprotectin than other groups. Fecal calprotectin was an effective early predictor of NEC in neonates with CHD with 90% sensitivity, and 92.5% specificity.

Keywords: Fecal Calprotectin; Congenital Heart Disease; Necrotizing Enterocolitis.

[a] Pediatrics Department, Faculty of Medicine Benha University, Egypt.

[b] Clinical and Chemical Pathology Department, Faculty of Medicine Benha University, Egypt.

Corresponding to:
Dr. Abeer E. Karish.
Pediatrics Department, Faculty of Medicine Benha University, Egypt.
Email: abeereisa92@gmail.com

Received:
Accepted:

Introduction

Congenital heart defects (CHDs)are a major public health problem: These defects lead to hospitalization and morbidity in kids, and frequently need lifelong monitore, They are the primary etiology of mortality in the 1st year of life, Collectively, congenital heart defects are prevalent, but individually they can correspond to uncommon or ultrarare disorders, needing expert identification and sophisticated repair operation [1] .

Necrotizing enterocolitis is an intestinal pathology which mostly influences preterm infants and term neonates with heart defects and is one of the most life-threatening disorders that influence neonates related to an elevated morbidity and death [2] , It occurs when an immature or impaired gastrointestinal tract permits bacteria to penetrate the mucosal barrier, leading to an inflammatory cascade which can result in ischemia and perforation [3] .

Neonates with ductal-dependent congenital heart disease or large left-to-right shunts are at greater possibility of mesenteric hypoperfusion [4].

It is most frequently observed in preterm infants, however it is not distinct to this population, congenital heart disease is a well-described risk factor for the progress of necrotizing enterocolitis in all preterm and term neonates [5]. In infants with normal birth weight or more than 2500 grams, congenital heart disease is documented to be implicated in up to eighteen percent [6].

NEC is a progressive disease which starts with feeding intolerance: elevated gastric residuals, abdominal distension, blood in the stool, and episodes of desaturation. The abdomen of the baby might be soft initially; but over time, peritonitis signs develop[7, 8].

Necrotizing enterocolitis is categorized based on it's severity utilizing the Bell staging criteria for necrotizing enterocolitis, which were first developed in 1978 and involved a set of features utilized to categorize infants into one of three stages of necrotizing enterocolitis, which were used to stratify infants by illness severity, then was modified by Walsh and Kliegman in 1986 through elevating the number of stages from three to six to guide therapeutic decisions depending on variances in illness severity across the prolonged stages. The newer staging system distinguished infants with Bell stage I through the features of bright red blood from the rectum (Stage IB) from those without this outcome (Stage IA). Furthermore, Stage IIA and IIB permitted distinguishing of illness severity, from infants who were mildly ill (Stage IIA) to moderately ill (Stage IIB) with ascites or portal venous gas. At the end, stage IIIB recognized infants with pneumoperitoneum, in contrast to stage IIIA[9].

Necrotizing enterocolitis is frequently identified by clinical symptoms as well as inflammatory markers and validated through intestinal radiographs. Nevertheless, the first clinical symptoms of necrotizing enterocolitis aren't specific and the illness development is quick. Currently, calprotectin was widely examined as a possible biomarker. Calprotectin is an antibacterial protein which is increased in the feces of inflammatory bowel disorders. Calprotectin is released into the lumen early in the illness, so it may be utilized for early identification of the necrotizing enterocolitis [10].

Neonates with congenital heart disease are at elevated possibility for suspected NEC because of reduced cardiac output, causing decreased mesenteric blood flow and gut ischemia. This hypoperfusion results in milk stasis, intestinal dilation, disrupted signal transduction, inflammation, and necrosis [11].

Abdominal radiography is a diagnostic instrument for necrotizing enterocolitis [12].

Fecal calprotectin: is a fecal biomarker which is utilized as a diagnostic indicator for necrotizing enterocolitis in preterm infants [13] .

Calprotectin (36.5 kDa) is a neutrophil activation indicator and is chiefly showed in the cytoplasm of neutrophils (approximately five percent of their total protein content) and expressed on activated macrophages and monocytes [14].
The goal of this work was to assess the role of fecal calprotectin in the early recognition of suspected NEC in infants with CHD.

Patients and Methods:

This case control study has been conducted on 80 preterm or full-term neonates, aged from 1 - 28 days old, both sexes, divided into 3 groups: group 1 (20 patients): CHD whether cyanotic or a cyanotic heart disease, group 2 (20 patients): CHD with suspected NEC (abdominal distention, gastric residual, bloody stool), NEC diagnosis was clinically, radiologically and using Bell's staging retrospectively, group 3 (40 subjects): control healthy neonates matched for gestational age and weight to other groups, admitted for weight gain or physiological jaundice at NICU department as neonates at outpatient visits it was not easy to take stool samples from them. Sample size calculated using epi info soft calculator version 3 based on **O'Connor et al., 2020,** study results the median (IQR)Fecal calprotectin level among patients with necrotizing enterocolitis and those without necrotizing enterocolitis were 3528 pg/mL (2417, 4641) and 390 pg/mL (56, 565) respectively. The power of the study 80%, 95% confidence level and alpha error 5%, so the minimal calculated sample size is 2 patients in each group and increased to twenty patients in each group for fear of dropout and defaulters.
The research has been done from April 2022 to April 2024 in Benha University Hospital following approval from the Ethical Committee Banha University Hospital, Banha, Egypt {M.S.26.4.2022}. An informed written consent has been attained from relatives of the cases.

Exclusion criteria were neonate with any gastrointestinal complication or malformation (such as gastroschisis or imperforate anus), neonates died or discharged before sample taking, and stool samples couldn't be obtained due to severe diarrhea or inadequate stool samples at point of analysis in laboratory.
All cases have been exposed to complete history taking, general, cardiac and abdominal examination, laboratory examinations [complete blood count (CBC), partial thromboplastin time (PTT), prothrombin time (PT), arterial blood gases (ABG) ,international normalized ratio (INR), C-reactive protein (CRP), electrolyte (Na, K, Ca, Mg), kidney function (urea and creatinine) and complete liver function (serum glutamic-pyruvic transaminase (SGPT), serum glutamic-oxaloacetic transaminase (SGOT) and albumin and radiological examination [X-ray ,US and echocardiogram].

Fecal calprotectin marker:

Stool sample collection:

Fecal calprotectin levels in diaper collected feces may be artificially increased through water absorption into the diapers or nappies. Also, fecal calprotectin levels may be influenced by bowel cleansing, and its concentrations can be increased due to the existence of blood in stool samples also for causes other than intestinal inflammation, such as anal fissures, haemorrhoids, so we avoided any contamination of stool samples by urine or blood as possible, also most of children received antibiotics so it is a potential confounder and there no available cases without antibiotics as most cases were admitted on antibiotics
(50 _100) mg stool samples collected in plastic containers, frozen to (-80 c) until batch analysis (every twenty-four to forty-eight hours), prior to analysis samples defrosted to room temperature, centrifuge samples for twenty minutes at 1000×g, eliminate particulates and assay immediately, or store samples in aliquots

at -20ºC or -80ºC. Prevent recurrent freeze/thaw cycles, the calprotectin has been determined utilizing commercially available enzyme-linked immunosorbent assay kit**,** the microtiter plate was provided in this kit had been pre-coated with an antibody specific to CALPRO. Standards or samples were then added to the appropriate microtiter plate wells with a biotin-conjugated antibody preparation specific to CALPRO. Next, Avidin conjugated to Horseradish Peroxidase (HRP) was added to each microplate well and incubated. After TMB substrate solution was added, only those wells that contained CALPRO, biotin-conjugated antibody and enzyme-conjugated Avidin exhibited a change in colour. The enzyme-substrate reaction was terminated by the addition of sulphuric acid solution and the colour change was measured spectrophotometrically at a wavelength of 450nm ± 10nm. The concentration of CALPRO in the samples was then determined by comparing the Optical density of the samples to the standard curve, Intra-assay Precision (Precision within an assay): 3 samples with low, middle and high level CALPRO were tested 20 times on one plate respectively, Inter-assay Precision (Precision between assays): 3 samples with low, middle and high level CALPRO were tested on 3 different plates, 8 replicates in each plate, CV(%) = SD/meanX100, Intra-Assay: CV<10%, Inter-Assay: CV<12%.

Lab analysis:

All studied neonates were tested for determination of stool calprotectin by using Human Calprotectin (CALPRO) ELISA Kit Catalog No: DL-CALPRO-Hu. Detection range: 31.2-2000pg/mL. Sensitivity: The minimum detectable dose of CALPRO is usually below 13.12pg/ml, specificity: no significant cross-reactivity or interference between CALPRO and analogues was observed

Statistical analysis:

Statistical analysis has been done by SPSS v27 (IBM©, Chicago, IL, USA). The Shapiro-Wilks test and histograms have been used to assess the normality of information distribution. Quantitative parametric data have been presented as average and standard deviation (SD) and have been analyzed by ANOVA (F) test with post hoc test (Tukey). Qualitative parameters have been presented as percentage (%) and incidence and have been analyzed utilizing the Chi-square test. Association between different parameters has been performed utilizing Pearson moment association equation. Multivariate regression was additional used to determine the association among a dependent parameter and more independent parameters. Roc curve has been used for evaluation of diagnostic performance specificity, sensitivity, negative predictive value (NPV) and positive predictive value (PPV). A 2 tailed P-value below 0.05 was regarded statistically significant.

Results:

Weight, SBP and DBP were significant reduced in the CHD + NEC group compared to the CHD and control groups (P-value below 0.05). An insignificant variance has been observed concerning gestational age, gender, maturity, twins, feeding type, time of starting feeding and length. Fenton growth charts the proportion of small for gestational age was significantly greater in the CHD +suspected necrotizing enterocolitis compared to the congenital heart disease and control groups (P-value below 0.05). HR, RR and temperature were significantly greater in the CHD + suspected necrotizing enterocolitis group in comparison with the congenital heart disease and control groups (P-value below 0.05). **Table 1**

Statistical echo among studied group were insignificantly different. **Table 2**

Abdominal X-ray findings and Bell's staging in patients with suspected NEC were enumerated in this table. **Table 3**

Table 1: General characteristics, vital signs and local examination of the studied groups

		CHD (number=20)	CHD + suspected NEC (number =20)	Controls (number= 40)	P
Sex	**Male**	9 (45.0%)	12 (60.0%)	19(47.5%)	0.577
	Female	11(55.0%)	8 (40.0%)	21(52.5%)	
Gestational age (weeks)		35.9 ±2	36.1 ±1.7	35.6 ±2.1	0.595
Maturity	**Preterm**	9 (45.0%)	7(35.0%)	11 (27.5%)	0.398
	Full term	11(55.0%)	13(65.0%)	29 (72.5%)	
Twins		2(10.0%)	3(15.0%)	3 (7.5%)	0.714
Feeding	**Breast**	1(5.0%)	4(20.0%)	8 (20.0%)	0.490
	Artificial	11(55.0%)	8(40.0%)	19 (47.5%)	
	Mixed	7(35.0%)	8(40.0%)	13 (32.5%)	
	NPO	1(5.0%)	0(0.0%)	0 (0.0%)	
Time of starting feeding	**Early**	17(85.0%)	14(70.0%)	29 (72.5%)	0.480
	Late	3(15.0%)	6(30.0%)	11(27.5%)	
Weight (kg)		2.3(1.2 - 4.5)	1.6 (1.2 - 5) [3]	2.5(1.6 - 3.5) [2]	**0.019***
Length (cm)		45 ±6	42 ±7	44 ±4	0.242
Fenton growth charts	**AGA**	2(10.0%)	0(0.0%)	19(47.5%)	**<0.001***
	LGA	4(20.0%)	4(20.0%)	0(0.0%)	
	SGA	14(70.0%)	16(80.0%)	21(52.5%)	
Vital signs	**HR (beats/m)**	140 ±17	149 ±9 [3]	138 ±3 [2]	**<0.001***
	RR (breaths/m)	46 ±8	50 ±7 [3]	42 ±3 [2]	**<0.001***
	SBP (mmHg)	81 ±16	72 ±9 [3]	83 ±4 [2]	**<0.001***
	DBP(mmHg)	51 ±18	45 ±9 [3]	52 ±7 [2]	**0.02***
	Temperature ℃	37.1 ±0.5	37.2 ±0.2 [3]	37 ±0.1 [2]	**0.016***

Information is presented as mean ± SD or median (IQR) or frequency (%). * Significant P-value; 1: Significantly different from the CHD group, 2: significantly different from the CHD+ suspected NEC group, 3: Significantly different from controls, CHD: Congenital heart disease, NEC: Necrotizing enterocolitis, NPO: Nothing per oral, AGA: Appropriate for gestational age, LGA: Large for gestational age, SGA: Small for gestational age, HR: Heart rate, RR: Respiratory rate, SBP: Systolic blood pressure, DBP: Diastolic blood pressure.

Table 2: Different type of CHD among studied groups

	CHD	CHD + suspected NEC	P
ASD	9(45.0%)	8(40.0%)	0.749
VSD	8(40.0%)	6(30.0%)	0.507
Hemodynamically significant PDA	3(15.0%)	8(40.0%)	0.077
F4	2(10.0%)	2(10.0%)	1.0
Cyanotic heart disease with lung plethora	4(20.0%)	4(20.0%)	1.0
Rt sided obstructive lesion	10(50.0%)	8(40.0%)	0.750
Lt sided obstructive lesion	4(20.0%)	2(10.0%)	0.372

ASD: Atrial septal defect, VSD: Ventricular septal defect, PDA: Patent ductus arteriosus, F4: Fallot, Cyanotic heart disease with lung plethora included: (DORV: Double outlet right ventricle, single ventricle), Right sided obstructive lesion included: (pulmonary stenosis, tricuspid atresia, pulmonary atresia), Left sided obstructive lesion included: (Aortic stenosis, Coarctation of the Aorta, Hypoplastic left heart syndrome).

Table 3: Abdominal X-ray findings and Bell's staging in patients with suspected NEC group

		N=22
Abdominal X-ray	**Dilated loops**	6(30.0%)
	Wall edema	2(10.0%)
	Free fluid	2(10.0%)
	Perforation	4(20.0%)
	Pneumatosis	8(40.0%)
		N=20
Bell's staging	**II-A**	4(20.0%)
	II-B	8(40.0%)
	III-A	4(20.0%)
	III-B	4(20.0%)

Data are presented as frequency (%). CHD: Congenital heart disease, NEC: Necrotizing enterocolitis.

Table 4: Faecal calprotectin and laboratory findings in the studied groups.

	CHD (number=20)	**CHD + suspected NEC (number = 20)**	**Controls (number= 40)**	**P**
Fecal calprotectin (pg/ml)	626.3 ±186.8 [2,3]	900 ±266.1 [1,3]	481.9 ±150 [1,2]	**<0.001***
Laboratory findings				
Hb (g/dL)	13.1 ±3	11.8 ±3.3 3	14.7 ±2.7 2	**0.002***
WBCS (cells/µL)	12.6 ±4.98	9.85 ±4.74	10.39 ±2.3	0.158
Platelets(cells/µL)	290 (156 - 451) [2]	190 (44 - 418) [1,3]	318 (189 - 411) [2]	**<0.001***
CRP (mg/L)	18 (5 - 91) [3]	29 (6 - 257) [3]	6 (3 - 14) [1,2]	**<0.001***
PH	7.35 ±0.04 [3]	7.33 ±0.19	7.38 ±0.03 [1]	**0.012***
PCO_2(mmHg)	39 ±9.2	36.6 ±13.6	37.1 ±2.9	0.646
HCO_3(mEq/L)	21.4 ±6.1	25.5 ±8.5	20.9 ±1.7	0.076
PT (seconds)	16.4 ±2.2 [3]	16.8 ±2.8 [3]	13.8 ±1.2 [1,2]	**<0.001***
INR	1.31 ±0.19 [3]	1.32 ±0.15 [3]	1.09 ±0.08 [1,2]	**<0.001***
Na(mEq/L)	136 ±3 [3]	134 ±7 [3]	139 ±4 [1,3]	**<0.001***
K(mEq/L)	4.8 ±1.2	4.7 ±1	4.3 ±0.8	0.188
Ca(mg/dL)	10.2 ± 9.07 [2, 3]	9.65 ± 8.92[1]	9.75 ± 8.52[1]	**0.133**
Urea(mg/dL)	26.5 (14.8 - 73)	45.5 (20 - 70) [3]	21.5 (4.4 - 29) [2]	**<0.001***
Creatinine(mg/dL)	0.73 ±0.36	0.63 ±0.18	0.53 ±0.11	0.059
SGOT(U/L)	21.5 (12 - 45)	19 (10 - 25)	20 (5.55 - 46)	0.262
SGPT (U/L)	26.5 (15 - 40)	27.5 (16 - 56)	19 (7 - 73)	0.051

HB: hemoglobin, WBCs: white blood cell, CRP: C reactive protein, PT: prothrombin time, INR: international normalized ratio, Na: sodium, K: potassium, Ca: calcium, SGPT: Serum Glutamic Pyruvic Transaminase, SGOT: serum glutamic-oxaloacetic transaminase.

Table 5: Outcome in the studied groups

	CHD (n=20)	**CHD + suspected NEC (n = 20)**	**P**
MV use	8 (40.0%)	10 (50.0%)	**0.750**
Need of inotropes	0 (0.0%)	4 (20.0%)	**0.113**
Length of NICU stay (day)	21 ±8 [3]	22 ±4 [3]	**0.620**
Mortality	8 (40.0%)	20 (100.0%)	**<0.001***

NICU: neonatal intensive care unit, MV: Mechanical ventilation.

Faecal calprotectin level, CRP, PT, INR and SGPT were significantly greater in the CHD + necrotizing enterocolitis group, followed by the congenital heart disease group and lowest in the control. HB levels, platelet counts, and Na levels were significantly reduced in the CHD + suspected necrotizing enterocolitis group in comparison with the congenital heart disease and control groups. An

insignificant variance has been observed between groups concerning WBCs, PCO2, HCO3, K, Ca, creatinine, SGOT, and SGPT. **Table 4**

An insignificant variance has been observed between groups regarding MV use, requirement of inotropes and length of NICU stay but a significant variance has been observed between groups regarding mortality (p-value below 0.05) **Table 5**

ROC analysis has been done for fecal calprotectin to differentiate between the CHD group and CHD with the NEC group. It revealed a significant excellent AUC of 0.805, with a 95% CI of 0.657 – 0.953 (P = 0.001). The best cutoff point was > 699 Pg/ml, at which sensitivity, specificity, PPV, and NPV were 80% for each. **Figure 1**

An insignificant association has been observed between faecal calprotectin and other parameters in the CHD group. In CHD+ suspected NEC group, a significant negative association has been observed among faecal calprotectin and weight (r = -0.449, P-value equal to 0.047) and creatinine (r = -0.454, P = 0.044), while other variables did not show significant correlations. **Table 6**

Multinomial logistic regression analysis has been performed to expect CHD+ suspected NEC using faecal calprotectin. Faecal calprotectin was a significant predictor of CHD with suspected NEC (OR = 1.011, 95% CI = 1.006 - 1.016, P-value below 0.001), controlling for gestational age, gender, weight, and type of feeding. **Table 7**

Table 6: Correlation between fecal calprotectin and other parameters in the CHD group and CHD+ suspected NEC group

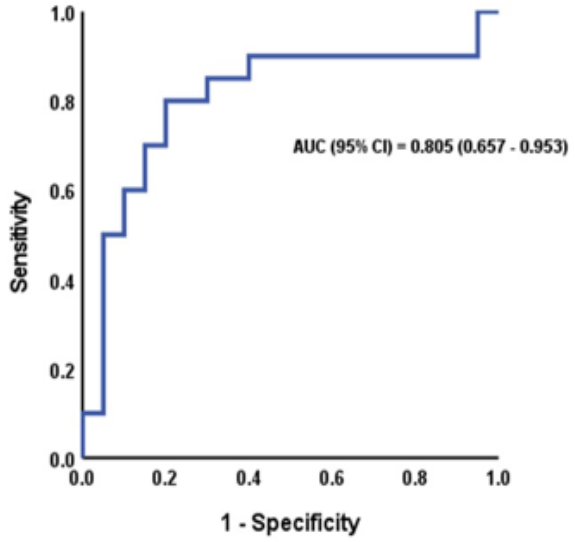
	Fecal calprotectin (Pg/ml)			
	CHD group		**CHD+ suspected NEC group**	
	R	**P**	**r**	**P**
Gestational age (weeks)	-0.176	0.457	-0.217	0.357
Weight (kg)	0.128	0.591	-.449	0.047
Length (cm)	-0.063	0.793	0.374	0.104
HR	-0.057	0.812	-0.312	0.18
RR	-0.211	0.372	0.049	0.837
SBP	0.145	0.542	-0.098	0.68
DBP	0.099	0.678	-0.044	0.854
Temperature	0.107	0.655	-0.041	0.864
HB	-0.015	0.952	0.682	**<0.001***
WBCS	0.221	0.35	-0.393	0.086
Platelets	-0.432	0.057	.539	**0.014***
PH	-0.422	0.064	0.129	0.589
PCO2	-0.144	0.544	0.043	0.856
HCO3	-0.283	0.226	.514	**0.021***
PT	0.256	0.276	0.429	0.059
INR	0.257	0.274	-0.415	0.069
Na	-0.2	0.397	-0.259	0.271
K	0.096	0.687	-0.32	0.170
Ca	0.131	0.583	-0.04	0.867
CRP	0.183	0.439	0.079	0.741
Urea	-0.038	0.874	.501	**0.024***
Creatinine	0.404	0.078	-0.179	0.451
SGOT	0.171	0.471	-.454	**0.044***
SGPT	0.123	0.605	0.178	0.453
Length of NICU stay (day)	0.276	0.239	-0.195	0.410

r: Correlation coefficient, * significant p value <0.05, PH: Potential of hydrogen, HCO_3: Bicarbonate, PCO_2: Partial pressure of carbon dioxide.

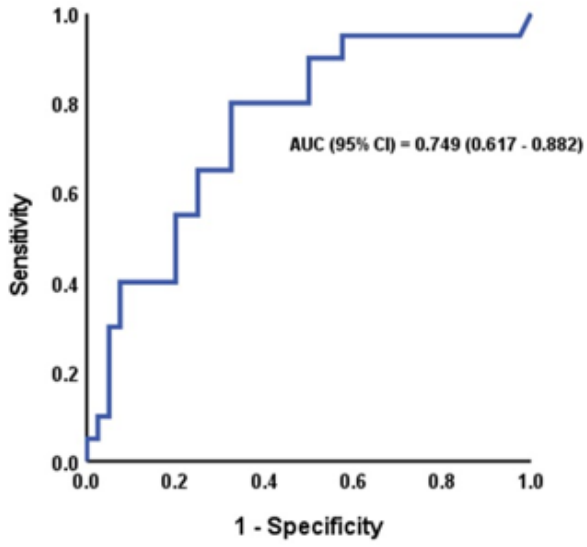
Table 7: Multinominal logistic regression analysis for faecal calprotectin to predict CHD+ suspected NEC

CHD + suspected NEC	OR (95% CI)	P
GA (weeks)	1.087 (0.658 - 1.795)	0.745
Sex	0.345 (0.065 - 1.839)	0.213
weight (kg)	1.445 (0.47 - 4.44)	0.52
Breastfeeding or artificial	1.675 (0.714 - 3.929)	0.236
Fecal calprotectin (Pg/ml)	1.011 (1.006 - 1.016)	**<0.001***

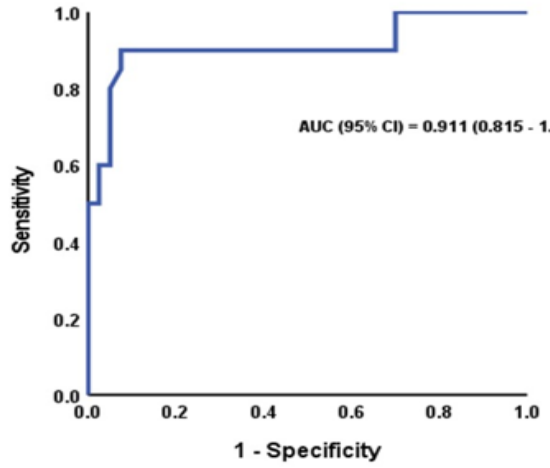
OR: Odds ratio; CI: Confidence interval, GA: gestational age.



(A)



(B)



(C)

Figure 1: ROC analysis for fecal calprotectin to differentiate between patients with (A) CHD only and those with congenital heart disease and suspected necrotizing enterocolitis, (B) cases with congenital heart disease only and controls, (C) patients with CHD with suspected NEC and controls as show in table 4.

Discussion

CHD is a structural anomaly of the heart present since birth. It is the most common congenital anomaly affecting 28% of all major congenital disabilities [15].

In the present study, comparable results were found between two groups of CHD whether NEC present or absent regarding subtypes of CHD or presence of murmur or cyanosis with statistically insignificant variance between subgroups of CHD in the 2 groups. Abdominal examination in the group of CHD with NEC revealed that abdominal distention observed in 100% of cases, Tender abdomen was found in 90% of cases. Diminished intestinal sounds were present in 30% of neonates in this group, also in this group, Bell's staging revealed that 40% were classified as stage II-B, 20% as stage II-A, 20% as stage III-A, and 20% as stage III-B. Abdominal radigraphic charecteristic finding revealed that 40% had pneumatosis, 30% showed dilated loops, 20% had perforation, 10% exhibited wall edema, and 10% presented with free fluid.

Chest examination and neurological examination were normal, showing no abnormalities across all groups.

Near to our findings, Lazow and colleagues [16] reported that abdominal distention was observed in 77.8 % of the CHD with suspected NEC group and inflammation of the abdomen was found in 11.1% and reported that abdominal X-ray findings revealed that 66.7% had pneumatosis in patients with CHD with suspected NEC and 11.1% had perforation. Supporting our findings, Spinner et al. [17] found that the cardiac lesions were comparable between suspected NEC group, and no suspected NEC group that included TOF.

Echo findings were comparable between the CHD and CHD + suspected NEC groups, including cardiac ASD, VSD, PDA, TOF, cyanotic heart disease (DORV), Right sided obstructive lesion, Left sided obstructive lesion.

In the current study, the mean Fecal calprotectin level (FCL) was highest in the CHD + suspected NEC group (900 ±266.1 pg/ml), followed by the CHD group (626.3 ±186.8 pg/ml), and lowest in the control group (481.9 ±150 Pg/ml).

Similar to our outcomes, Liu et al. [18] found that the suspected NEC patients showed significantly higher FC levels with a median value of 521.56 than the control group with a median value of 213.34. Supporting our findings, O'Connor et al. [19] and Emil Eskander et al. [20] and Abdelkader et al. [21] documented that the median FCL was higher in the suspected NEC group (3528, 167 and 875) compared to no suspected NEC group (390 and 69) respectively.

Our findings may be explained by that the mean fecal calprotectin level is highest in neonates with cardiogenic NEC likely due to increased intestinal inflammation caused by compromised blood flow. Cardiac issues can lead to reduced gut perfusion, resulting in ischemia and mucosal damage, which triggers a strong inflammatory response and elevates calprotectin levels.

In our study, laboratory findings, Haemoglobin (HB), and Platelet count levels were reduced in the CHD + NEC group in comparison with the CHD and control groups. C-reactive protein (CRP) concentrations were highest in the CHD + NEC group in comparison with the CHD and control groups. pH levels were slightly reduced in the CHD + NEC group in comparison with the CHD and control groups. Prothrombin time (PT) was longer in the CHD + NEC group in comparison with the CHD and control groups, and the international normalized ratio (INR) followed a similar pattern in CHD + NEC vs in CHD and controls. Sodium (Na) levels were reduced in the CHD + NEC group in comparison with the CHD and control groups. Urea concentrations were significantly greater in the CHD + NEC group in comparison with the CHD and control groups. Alanine aminotransferase

(SGPT) was significantly greater in the CHD + necrotizing enterocolitis group in comparison with the control group. An insignificant variance was observed regarding calcium (Ca), WBCs, PCO_2, HCO_3, K, creatinine, SGOT, and SGPT.

Our findings may be explained by that CHD and NEC can lead to increased stress on the body, resulting in haemolysis or blood loss. Additionally, NEC can cause inflammation and affect nutrient absorption, leading to deficiencies in iron and other essential components for red blood cell production. . [22]

In agreement with our results about laboratory findings, O'Connor et al. [19] showed that CRP concentrations were higher in suspected NEC compared to no suspected NEC.

Also, Emil Eskander and researches [20] reported that CRP was greater in suspected NEC group in comparison with control group. Hb, platelet count, and NA levels were reduced in the suspected NEC group compared to the control group.

In our research, regarding outcome, mechanical ventilation (MV) was required in 40% of the CHD group and 50% of the CHD + suspected NEC group. The need for inotropes was present in 20% of the CHD + suspected NEC group but not needed for the CHD group. As NEC is systemic condition leads to septic shock and multiorgan dysfunction and abdominal distension, both of which cause a significant decrease in gas exchange, leading to the need for MV and inotropes. The length of NICU stay was insignificantly different among the congenital heart disease and CHD + suspected NEC groups. Mortality was 40% in the congenital heart disease group and 100% in the CHD + suspected necrotizing enterocolitis group, with a significant difference observed between both groups, as all neonates in this group were critically ill, accompanied by severe thrombocytopenia and hemodynamic instability which lead to death.

Nearly, O'Connor et al. [19] showed that the length of NICU stay was significantly longer in suspected NEC (28 ± 10 days) compared to no suspected NEC (18 ± 11 days). However, four patients died (13%), of which two did not have suspected NEC. This was also consistent with Kessler et al. [23] who observed that cases with necrotizing enterocolitis accompanied with congenital heart disease had longer hospitalization and a higher death rate was seven fold higher than that of cases with necrotizing enterocolitis. Moreover , Pickard et al. [4] showed that infants with congenital heart disease had superior short-and long-term outcomes.

Furthermore, Bubberman et al. [24] reported that MV was required in 67% of the congenital heart disease + suspected NEC group and 81% in the preterm- suspected NEC group. The need for inotropy pre-suspected NEC was present in 28% of the CHD + suspected NEC group and 25% preterm- suspected NEC group. The need for inotropy peri-NEC was present in 6% of the CHD + NEC group but not needed for preterm- suspected NEC group. Mortality was 25% in the preterm-suspected NEC group and 39% in the CHD + suspected necrotizing enterocolitis group.

In the current research, ROC analysis has been performed for fecal calprotectin to differentiate between the CHD group and CHD with suspected NEC group. It revealed a significantly excellent AUC of 0.805. The best cutoff point was > 699 Pg/ml, at which sensitivity, specificity, PPV, and NPV were 80% for each.

ROC analysis was done for fecal calprotectin to differentiate between CHD + suspected NEC group and the control group. It revealed a significantly excellent AUC of 0.911, The best cutoff point was >667.1, at which 90%, sensitivity, 92.5%, specificity, 85.7%, PPV, and 92.5%, NPV.

Near to our findings, Liu et al. [18] found that for fecal calprotectin to differentiate between suspected NEC group and the

control group with 67.5% specificity, 76.7% sensitivity, 63.9% positive predictive value, and 79.4% negative predictive value.

Also, Emil Eskander et al.[20] reported that for fecal calprotectin to differentiate between suspected NEC from other conditions with 100% sensitivity, 100% specificity, and AUC of 0.994.

In our study, fecal calprotectin was positively correlated with WBCs, PCO_2, and CRP. Additionally, there was a strong positive correlation with Bell's staging. Negative correlations were found with weight and creatinine.

In the same line with our findings, Romih and colleagues [25] showed that there was a significant negative association between fecal calprotectin levels before and after feeding with weight.

Supporting our findings, O'Connor and researchers [19] showed that fecal calprotectin was negatively correlated with weight. While there was no association between fecal calprotectin concentrations and CRP. In our study, multinomial logistic regression analysis revealed that fecal calprotectin was a significant predictor of CHD with suspected NEC, controlling for gestational age, sex, weight, and type of feeding.

Like our findings, Liu et al. [18] reported that fecal calprotectin levels are valuable for early suspected necrotizing enterocolitis recognition.

Limitation of the research involved that the size of sample was relatively small. The research was in a single center. All studied neonate received antibiotics so, we were not able to recognize impact of postnatal antibiotics on FCP level so it is a potential confounder and there no available cases without antibiotics.

We didn't assess diarrhea and bleeding that can lead to false negative results. We didn't assess the effect of urine in diaper as it may give false negative results. We couldn't study effect of types of CHD (non-cyanotic and cyanotic) or level of ventricle dysfunction on level of FCP.

Conclusions:

Neonate with CHD and suspected NEC had significantly elevated level of fecal calprotectin than patients with congenital heart disease only and control. Fecal calprotectin was an effective early predictor of suspected NEC in patents with CHD with 90% sensitivity, and 92.5% specificity.

Conflict of interest:

None of the contributors declared any conflict of interest.

References:

1. Houyel L, Meilhac SM. Heart development and congenital structural heart defects. Annu Rev Genomics Hum Genet. 2021;22(2):257-84.
2. Schaan CW, Feltez G, Schaan BD, Pellanda LC. Functional capacity in children and adolescents with congenital heart disease. Rev Paul Pediatr. 2019;37(1):65-72.
3. Fisher JG, Bairdain S, Sparks EA, Khan FA, Archer JM, Kenny M, et al. Serious congenital heart disease and necrotizing enterocolitis in very low birth weight neonates. J Am Coll Surg. 2015;220(6):1018-26.e14.
4. Pickard SS, Feinstein JA, Popat RA, Huang L, Dutta S. Short- and long-term outcomes of necrotizing enterocolitis in infants with congenital heart disease. Pediatr. 2009;123(5):e901-6.
5. Motta C, Scott W, Mahony L, Koch J, Wyckoff M, Reisch J, et al. The association of congenital heart disease with necrotizing enterocolitis in preterm infants: a birth cohort study. J Perinatol. 2015;35(11):949-53.
6. Velazco CS, Fullerton BS, Hong CR, Morrow KA, Edwards EM, Soll RF, et al. Morbidity and mortality among "big" babies who develop necrotizing enterocolitis: a prospective multicenter cohort analysis. J Pediatr Surg. 2018;53(1):108-12.
7. Glasser JG. Abdominal compartment syndrome complicating necrotizing enterocolitis: A case report. Ann Med Surg (Lond). 2021;71:102961.
8. Sosa PA, Firnberg M, Tsung JW. Point-of-care ultrasound evaluation of suspected necrotizing enterocolitis in the ED. Am J Emerg Med. 2024;76(2):270.e1-.e4.
9. Patel RM, Ferguson J, McElroy SJ, Khashu M, Caplan MS. Defining necrotizing enterocolitis: current difficulties and future opportunities. Pediatr Res. 2020;88(1):10-5.
10. Qu Y, Xu W, Han J, Zhou W, Wu H. Diagnostic value of fecal calprotectin in necrotizing enterocolitis: A meta-analysis. Early Hum Dev. 2020;151(2):105170.

11. Neu J, Walker WA. Necrotizing enterocolitis. N Engl J Med. 2011;364(3):255-64.
12. Gephart SM, Gordon PV, Penn AH, Gregory KE, Swanson JR, Maheshwari A, et al. Changing the paradigm of defining, detecting, and diagnosing NEC: Perspectives on Bell's stages and biomarkers for NEC. Semin Pediatr Surg. 2018;27(1):3-10.
13. Pergialiotis V, Konstantopoulos P, Karampetsou N, Koutaki D, Gkioka E, Perrea DN, et al. Calprotectin levels in necrotizing enterocolitis: a systematic review of the literature. Inflamm Res. 2016;65(11):847-52.
14. Yui S, Nakatani Y, Mikami M. Calprotectin (S100A8/S100A9), an inflammatory protein complex from neutrophils with a broad apoptosis-inducing activity. Biol Pharm Bull. 2003;26(6):753-60.
15. Singh R, Jain P, Dhakar J, Shrivastava M, Gohiya P, Thakur S, et al. Clinical profile and pattern of congenital heart disease in neonates and immediate outcome based on oxygen saturation. IJLSSR. 2024;10(2):12-5.
16. Lazow SP, Tracy SA, Estroff JA, Parad RB, Castro-Aragon IM, Fujii AM, et al. A role for abdominal ultrasound in discriminating suspected necrotizing enterocolitis in congenital heart disease patients. Pediatr Surg Int. 2022;38(2):225-33.
17. Spinner JA, Morris SA, Nandi D, Costarino AT, Marino BS, Rossano JW, et al. Necrotizing enterocolitis and associated mortality in neonates with congenital heart disease: A multi-institutional study. Pediatr Crit Care Med. 2020;21(3):228-34.
18. Liu S, Liu Y, Lai S, Xie Y, Xiu W, Yang C. Values of serum intestinal fatty acid-binding protein, fecal calprotectin, and fecal human β-defensin 2 for predicting necrotizing enterocolitis. BMC Pediatr. 2024;24(1):183.
19. O'Connor G, Brown KL, Taylor AM. Faecal calprotectin concentrations in neonates with CHD: pilot study. Cardiol Young. 2020;30(5):624-8.
20. Emil Eskander A, Hosni Tomerak R, Safwat M, Ghobrial C, Nabih Almohammady M, Abdelfattah W. Fecal calprotectin: A screening marker for the early detection of necrotizing enterocolitis among children in egypt. Iran J Neonatol. 2020;11(2):1-9.
21. Abdelkader M, Mesbah BE-D, Khashana A. Fecal calprotectin level in neonates with necrotizing enterocolitis. Iran Jl of Neonatol. 2019;10(3):7-13.
22. Roychaudhuri, S., Grewal, G., Vijayashankar, S. S., Lavoie, P. & Maheshwari, A. Necrotizing enterocolitis associated with congenital heart disease-a review article. Newborn (Clarksville). 2022; 1(1): 170-6.
23. Kessler U, Hau E-M, Kordasz M, Haefeli S, Tsai C, Klimek P, et al. Congenital heart disease increases mortality in neonates with necrotizing enterocolitis. Front Pediatr. 2018;6:312.
24. Bubberman JM, van Zoonen A, Bruggink JLM, van der Heide M, Berger RMF, Bos AF, et al. Necrotizing enterocolitis associated with congenital heart disease: A different entity? J Pediatr Surg. 2019;54(9):1755-60.
25. Romih MM, El-hindawy EM, Khalifaa NA, Othman AM. Fecal calprotectin level in term and preterm babies before and after starting of feeding. J Egypt Public Health Assoc. 2021;82(4):733-9.

To cite this article: Somaia A. Elwan, Abeer E. Karish, Maha M. Osman, Eman G. Abdelrahman. Study the Value of Fecal Calprotectin in Neonate with Congenital Heart Disease and Necrotizing Enterocollitis. BMFJ XXX, DOI: 10.21608/bmfj.2025.378475.2381.