The diagnostic accuracy of fecal calprotectin in necrotizing enterocolitis in neonates in Aswan Specialized Hospital

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ABSTRACT

Background: Necrotizing enterocolitis (NEC) is the most common acquired multifactorial gastrointestinal emergency that primarily affects pre-term infants. It's a fatal condition, e specially in low-birth-weight neonates.

Objective: To assess the value of fecal calprotectin as an early non-invasive indicator for the diagnosis of NEC.

Patients and Methods: This is a case-control cross-sectional study that included 50 neonates admitted to the NICU in Aswan Specialized Hospital who suffered from NEC, compared to 50 completely healthy neonates as a control group born between May 2021 and October 2022. All infants enrolled in this study were subjected to through history taking, complete clinical examination, and laboratory investigations including; (CBC, CRP. ABG, serum sodium level fecal calprotectin), and radiological imaging (plain erect abdominal x-ray and abdominal US). A small amount of stool (0.5gm) was needed to detect calprotectin concentration in the fecal sample using ELISA.

Results: We found that there was a significant correlation between the disease WBCs and CRP. Moreover, fecal calprotectin showed a significant difference between diseased cases and control. Also, there was no correlation between fecal calprotectin and post-natal age, gestational age, or sex. However, there's a strong significant positive correlation between fecal calprotectin and CRP in all the diseased cases. Fecal calprotectin among the control group was 20 (17-29.25) mg/gm stool and among the case group was 198.5 (186- 240.75).mg/gm stool

Conclusion: We concluded that fecal calprotectin can be used as a very useful diagnostic biomarker for NEC especially in the early stage of the disease in both preterm and full-term where the clinical, laboratory, and radiological investigations could be non-specific

Keywords: Necrotizing Enterocolitis, Calprotectin, Preterm, NICU.

INTRODUCTION

Necrotizing Enterocolitis (NEC) is a serious inflammatory disorder that is characterized by intestinal necrosis with sufficiently great morbidity and mortality rates in the neonatal intensive care units (NICU) [1].

It affects about 1-5% of newborns in the NICU, especially premature and low birth weight infants, continuous advances have been achieved in the care of critically ill neonates; however, the complications and mortality rates of NEC have remained unchanged [2].

Abnormal inflammatory response by the newborn intestine to luminal microbes is the main theory for NEC pathogenesis [3].

The immune system in neonates is vulnerable Basu et al. [4] which disrupts the pro-inflammatory and anti-inflammatory mediator balance; moreover, the dysbiosis in gut microbiota can contribute to the disease occurrence [5].

The NEC has three clinical stages according to Bell's staging system [6]**.** The classic form usually presents with feeding intolerance, abdominal distension, and blood in the stool. Complications appear in the form of abdominal discoloration and intestinal perforation that may lead to peritonitis [7]**.** Many preventive measures have been described [8]**,** however, the prognosis of NEC is generally poor owing to its rapid onset and progression to death, as well as its morbidity when the infant survives [9].

The diagnosis of NEC remains challenging since the initial presentation is nonspecific with no single reliable laboratory tests or imaging tools Therefore, it is mandatory to search for highly sensitive and specific biomarkers for the screening of NE [10].

Calprotectin is one of more than 20 proteins in the so-called S-100 family that forms about 60% cytosolic protein in neutrophils. It has antimicrobial activity as an antifungal and antibacterial agent [11].

It inhibits metalloproteinase and chelates with zinc and manganese ions since it is proposed to inhibit microbial proliferation [12].

During intestinal inflammation, neutrophils migrate towards the gut and are sequestered into its wall. Following that, calprotectin is released from activated neutrophils into the intestinal lumen and exhibits pro-inflammatory properties [13].

Its amount in stool reflects the movement of neutrophil into the gut lumen; therefore, it is a good indicator of the severity of inflammation [14].

Calprotectin resists heat, proteolysis, and degradation by bacteria in the gut. Moreover, it participates in the diagnostic workup of neonates suspected to have inflammatory bowel disease [15].

Fecal calprotectin has been extensively studied in NEC, and its concentration was found to be elevated in newborns with NEC, compared to healthy neonates. Its levels appeared to decline after the treatment initiation, which may highlight its role as a potential marker for monitoring response to therapy [16].

However, it is not known yet whether high calprotectin concentrations at the beginning of bowel symptoms recognize neonates with true NEC versus other bowel disorders.

Accordingly, its significance as an early screening marker remains unknown. Moreover, no definite cut-off values have been settled for screening in early bowel symptoms [17]. Thus, it is hypothesized that measuring its level early in NEC could help as a screening marker.

AIM OF THE WORK

This study aims to assess the fecal calprotectin value as a recent marker and to examine its characteristics as a non-invasive diagnostic marker for NEC (Necrotizing Enterocolitis) diagnosis in neonates.

PATIENTS AND METHODS

Ethical consideration

A verbally informed consent was obtained from mothers before the study.

The approval of the local ethical committee was obtained before the study.

Confidentiality and personal privacy were maintained throughout all stages of the research**.**

Competing Interests

The authors declare no competing interests.

Funding

There was no fund.

Data availability

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Inclusion criteria

1. All newborn babies of gestational age are more than or equal to 28 weeks.
2. All neonates weigh between 1Kg to 3.5 Kg
3. All neonates were admitted to Aswan specialized hospital at the time of the study.

Exclusion criteria

1. All neonates with gestational age less than 28 weeks.
2. All neonates weigh less than 1Kg or more than 3.5 Kg.
3. If the baby isn't admitted to Aswan specialized hospital

Participants and the study design

The study was conducted on 100 neonates born between May 2021 and October 2022 who were admitted at Aswan specialized hospital (50 sick newborn babies who had NEC., and 50 healthy neonates who were admitted due to other causes as monitoring serum blood glucose in infants of diabetic mother or for weight gain). All the studied neonates were subjected to the following:

Complete history intake, including prenatal history, maternal disease and drugs, perinatal history, and history of last menstrual date.

Ballard score for estimation gestational age, anthropometric assessments, and a systemic examination, including admission and treatment plan.

Full clinical examination including

The following investigations were done to all babies in this study:

CBC Withdrawal of 1ml whole blood on EDTA. By sysmex and swelab alpha machine showing leucopenia, anemia, or thrombocytopenia according to the age and sex normal varieties in the case group.

Quantitative estimation of CRP in the case group. A serum sample was obtained on AVITEX CRP by Rapid latex agglutination test kit.

ABG: 2-3 ml arterial specimen collected from a peripheral artery in a 3- or 5- ml plastic or glass airtight syringe fitted with a small-bore needle. Heparin must be added to the syringe as an anticoagulant. And then the specimen was analyzed with no delay. showed metabolic acidosis in the case group.

Serum sodium

Fecal calprotectin:

Sample preparation:

50 -100 mg Stool samples were collected with a disposable, breakable inoculation loop (10 Ml, sterile, firm loop, T572-B, Technical Service Consultants, and Lancaster, UK) and entered into a 14- ml disposable screw cap tube (Greiner tube, HGreiner GmbHA, Labortechnik, Frickenhausen, Germany). The feces weight was measured and the loop handle was immediately broken off. Extraction solution containing urea and citrate (Nycomed Pharma AS).was added in a ratio of 1:50. After 30 s agitation on a mixer (Whirli Mixer ™, Fisons Scientific Equipment, UK) followed by homogenization for 20 min at 1400 rPm on a shaker.1 ml of this homogenate was transferred to an Eppendorf tube and centrifugated for 20 min at 10,000 ×g. The supernant (0.5 ml) was collected immediately and stored frozen at -20c for later analysis.

Determination of fecal calprotectin

The supernants of all fecal sample extractions had been assayed in a calprotectin ELISA (PhiCal ELISA kit,Nycomed Pharma AS) with standards and controls included and performed according to the manufacturer's instructions. The supernants then were diluted 12-2000-fold in the kit dilution liquid. After that, those diluted supernants (100ML) were added to the microtiter plate wells, and incubated at room temperature for about 45 min. on a shaker (400 rPm). And washed 4 times with washing solution.

Later, the affinity-purified rabbit anti-calprotectin antibodies were conjugated with alkaline phosphatase (100 ml/well) added and incubated for 12 min at room temperature on a shaker (400 rPm). After washing the wells 4 times, enzyme substrate was added and optical densities read at 630 nm. Calprotectin concentrations were calculated from the standard curve obtained with the kit standards. At last, the 100-ML of LISA stop solution was added into each of the wells and mixed gently, the absorbance was red at 450 nm with reference filter at 630 nm.

Concentration of fecal calprotectin

In our study, the cut-off value was 140 µg/g stool in the case group which is the quadrable of the highest fecal calprotectin level in the control group).

B-Radiological study: plain erect x-ray and abdominal ultrasound.

Statistical analysis

SPSS version 20.0 was used to analyze the data (SPSS Inc., Chicago, Il, USA). Continuous variables having a normal distribution were reported as mean, standard deviation, and numbers and percentages, respectively. Also, numerical data was equated by the Chi-square, independent t-test or Mann- Whitney U test. Then one- way analysis of the variance (ANOVA) was used to compare continuous variables, and $p > 0.05$ was considered to be significant statistically.

RESULTS.

Table (1): Comparison between Socio-demographic characteristics in case and control groups

	Cases (n=50)	**Control (n=50)**	
Age (days)			
Range	3 – 15	3 – 5	
Mean±SD	6.64±2.36	3.66±0.72	
Median (IQ range)	6(5 - 8)	4(3 - 4)	**<0.001****
Gender			
Male	27(54%)	26(52%)	0.841
Female	23(46%)	24(48%)	
Gestational age (weeks)			
Range	30 – 37	34 – 39	
Mean ±SD	32.64±1.72	37.28±1.31	
Median (IQ range)	32(31 - 34)	37(37 - 38)	**<0.001****
Pre-term	48(96%)	4(8%)	**<0.001****
Full-term	2(4%)	46(92%)	
Mode of delivery			
NVD	1(2%)	9(18%)	
CS	49(98%)	41(82%)	
Weight (kg)			
Range	1 - 3.5	1.8 - 3.51	
Mean±SD	1.53±0.42	3.1±0.45	
Median (IQ range)	1.4(1.28 - 1.75)	3.2(3 - 3.4)	

There were significant differences between the cases-group and control group regarding socio-demographic characteristics regarding postnatal age, gestational age, mode of delivery and weight as shown in Table 1.

Table (2): Comparison between case and control groups regarding clinical data

	Cases (n=50)	Control (n=50)	P. value
RR (C/M)			
Range	62 – 86	36 – 51	
Mean±SD	69.74±5.5	41.48±3.22	
Median (IQ range)	70(65 - 73.25)	41(39 - 44)	**<0.001****
HR (B/M)			
Range	141 – 200	115 – 137	
Mean±SD	162.3±10.85	124.64±6.68	
Median (IQ range)	161.5(155 - 169)	121.5(119 - 131.25)	**<0.001****
Mean ABP (mmHg)			
Range	58 – 70	60 – 69	
Mean±SD	62.16±2.39	60.82±1.41	
Median (IQ range)	61.5(60 - 64)	61(60 - 61)	**0.001****
Temp. (°C)			
Range	36 – 38	36 – 37.3	
Mean±SD	36.24±0.41	36.72±0.41	
Median (IQ range)	36.2(36 – 36.3)	36.65(36.3 – 37.2)	**<0.001****

There was significant increase in RR, HR, ABP in cases group than in control group while temperature in cases group was lower than in control. The mean ± SD RR in cases group was 69.74±5.5 (C/M) and 41.48±3.22 (C/M) in control group. The mean ± SD HR in cases group was 69.74±5.5 B/M and 41.48±3.22 B/M in control group. The mean ± SD ABP in cases group was 69.74±5.5 mmHg and 41.48±3.22 mmHg in control group. Temp. ranged between 36 – 38 °C in cases group and 36– 37.3 °C in control group. (Table 2)

Table (3): Comparison between case and control groups according to laboratory data

	Cases (n=50)	Control (n=50)	P. value
TLC (10^3)			
Range	11 – 30	5 – 9	
Mean±SD	23.64±4.77	8.03±1.03	
Median (IQ range)	22.5(20 - 28.25)	8.5(7.48 - 8.9)	**<0.001****
Neutrophils%			
Range	45 – 87	50 – 67	
Mean±SD	63.42±9.32	54.24±3.83	
Median (IQ range)	61(57 - 69.25)	55(51 - 55.25)	**<0.001****
Lymphocytes%			
Range	18 – 47	30 – 46	
Mean±SD	33.3±8.05	38.6±4.76	
Median (IQ range)	34.5(27.75 - 39)	39(35.75 - 42)	**<0.001****

Plat. (10^3)			
Range	23 – 120	290 – 450	
Mean±SD	68.4±21.81	372.54±48.77	
Median (IQ range)	70.5(51 - 85.25)	365.5(331.5 - 411)	**<0.001****
Hb. (gm/dl)			
Range	7 - 10.7	12 – 14	
Mean±SD	9.37±0.88	12.61±0.45	
Median (IQ range)	9.55(8.95 - 10)	12.6(12 - 13)	**<0.001****
Na (m Eq/L)			
Range	114 – 131	135 – 143	
Mean±SD	121.16±5.27	138.9±1.91	
Median (IQ range)	120(117 - 125.5)	139(137.75 - 140)	**<0.001****
CRP (mg/dl)			
Range	10 – 69	1 – 4	
Mean±SD	49.1±14.98	2.38±1.23	
Median (IQ range)	50(40 - 60.25)	2(1 - 4)	**<0.001****

According to laboratory data, there was significant different between cases and control groups. The mean ± SD level of TLC (10^3) was 23.64±4.77 in cases group and 8.03±1.03 in control group. The mean ± SD level of Neutrophils% was 63.42±9.32 in cases group and 54.24±3.83 in control group. The mean ± SD level of Lymphocytes% was 33.3±8.05in cases group and 38.6±4.76 in control group. The mean ± SD level of Plat. (10^3) was 68.4±21.81 in cases group and 372.54±48.77 in control group. Hb. ranged between 7 - 10.7 gm/dl in cases group and 12 – 14 gm/dl in control group. The mean ± SD level of Na was 68.4±21.81 m Eq/L in cases group and 372.54±48.77 m Eq/L gm/dl in control group. CRP level ranged between 10 – 69 in cases group and 1 – 4 in control group. (Table 3)

Table (4): Multivariate regression analysis of risk factors of NEC and fecal calprotectin among studied patients

	Beta	**P. value**	**95.0% Confidence Interval for B**	
			Lower	**Upper**
Weight (kg)	-0.234	0.091	-45.732	3.533
Apgar score	-0.041	0.770	-7.870	5.862
RR (C/M)	0.170	0.193	-0.618	2.982
Plat. (10^3)	-0.405	**0.003****	-1.173	-0.248
CRP (mg/dl)	0.123	0.336	-0.336	0.963

Regarding multivariate regression analysis, only platelets were the risk factors of NEC and fecal calprotectin. (Table 4)

Table (5): Comparison between cases and control groups according to fecal calprotectin

	Cases (n=50)	Control (n=50)	P. value
Fecal Calprotectin			
Range	140 – 277	15 – 34	
Mean±SD	206.44±38.24	22.82±6.37	
Median (IQ range)	198.5(186 - 240.75)	20(17 - 29.25)	**<0.001****

The mean level of fecal calprotectin was significantly increased in cases group (206.44) than in control group (22.82). (Table 5)

Table (6): Comparison between fecal calprotectin in pre-term and full-term patients

	Pre-term (n=48)	Full-term (n=2)	P. value
Fecal Calprotectin			
Range	140 – 277	140 – 140	
Mean±SD	209.2±36.45	140±0	
Median (IQ range)	199(188.25 - 242.25)	140(140 - 140)	**0.023***

P value 0.05 statistically significant SD = standard deviation IQ = interquartile range.

The level of fecal calprotectin was significantly increased in pre-term patients with a mean ± SD of 209.2±36.45 than in full-term patients (140±0). (Table 6)

Table (7): Correlation coefficient analysis of fecal calprotectin with other parameters among studied patients (n=50).

Fecal Calprotectin		
	R	P
Age (days)	-0.086	0.551
Gestational age(weeks)	-0.094	0.514
Weight (kg)	**-0.286**	**0.044***
Apgar score	**-0.280**	**0.049***
RR (C/M)	**0.298**	**0.036***
HR (B/M)	0.011	0.937
Mean ABP (mm Hg)	0.012	0.933
Temp.(°c)	-0.063	0.662
TLC (10^3)	0.175	0.223
Neutrophils%	0.194	0.177
Lymphocytes%	-0.243	0.089
Plat. (10^3)	**-0.511**	**0.000****
Hb. (gm/dl)	-0.177	0.220
Na (m Eq/L)	-0.120	0.405
CRP (mg/dl)	**0.285**	**0.045***

Regarding correlation between fecal calprotectin and other parameters, there was a significant negative correlation between weight, Apgar score, RR, Plat. (10^3), CRP and fecal Calprotectin. While, no significant correlation between age, gestational age, HR, and mean ABP Temp., TLC (10^3), Neutrophils%, Lymphocytes%, Hb., Na and fecal Calprotectin. (Table 7)

Table (8): ROC curve analysis of NEC between case and control groups according to the studied parameters

	AUC	Cut off	Sensitivity	Specificity	PPV	NPV	Accuracy
Weight (kg)	0.969	≤2.5	98	89.58	90.7	97.7	93.8
Apgar score	0.970	≤9	94	100	100	94.3	97
RR (C/M)	1.0000	>51	100	100	100	100	100
Fecal calprotectin	1.000	>34	100	100	100	100	100
TLC (10^3)	1.000	>9	100	100	100	100	100
Neutrophils%	0.827	>56	78	92	90.7	80.7	85
Lymphocytes%	0.713	≤31	46	88	79.3	62	67
Plat. (10^3)	1.000	≤120	100	100	100	100	100
Hb. (gm/dl)	1.000	≤10.7	100	100	100	100	100
Na (m Eq/L)	1.000	≤131	100	100	100	100	100
CRP (mg/dl)	1.000	>4	100	100	100	100	100

Regarding ROC curve analysis of NEC between case and control groups, weight showed a sensitivity of 98%, specificity of 89.58%, PPV of 90.7% and NPV of 97.7%, whereas Apgar score showed a sensitivity of 94%, specificity 100%, PPV 100% and NPV 94.3% compared to RR that showed a sensitivity of 100%, specificity of 100%, PPV of 100% and NPV of 100%. Fecal calprotectin showed a sensitivity of 100%, specificity of 100%, PPV of 100% and NPV of 100%. TLC showed a sensitivity of 100%, specificity of 100%, PPV of 100% and NPV of 100%. Neutrophils% showed a sensitivity of 78%, specificity of 92%, PPV of 90.7% and NPV of 80.7%. Lymphocytes% showed a sensitivity of 46%, specificity of 88%, PPV of 79.3% and NPV of 62%. Plat., Hb., Na, CRP showed a sensitivity of 100%, specificity of 100%, PPV of 100% and NPV of 100%. (Table 8)

Discussion:

Necrotizing enterocolitis is a common and possibly fatal inflammatory disorder that is seen typically among preterm neonates. It's the most common gastrointestinal emergency in neonates. It's characterized by variable damage to the intestinal wall all over the intestinal tract, ranging from mild injury to the intestinal mucosa to full-thickness necrosis and intestinal perforation which can lead to sepsis and even death.

In our study, fifty neonates (27 boys and 23 girls) were included who suffered from NEC and were admitted at NICU at Aswan Specialized Hospital. Two of the cases were full-term and 48 were pre-term with postnatal age ranging from 3-15 days. with a statistically significant higher level of fecal calprotectin among pre-term patients (209.2 +/- 36.45 m/g feces) compared with full-term patients (140 +/- 0 m/g feces). (p= 0.023).

Additionally, in 2014, Yoon et al. [18] analyzed the correlation between fecal calprotectin level in the preterm babies not having NEC and the number of days after birth, with coefficient - 0.171, showing a statistical significance (p= 0.04), despite that when interpretation for these coefficient values done, both of the 2 variables showed a weak negative relationship, and after data presentation by a scatter diagram, the two variables had no

relationship, so it was very difficult to clarify the change tendency in the levels of fecal calprotectin.

In this study, there was no significant correlation between neither the age (r = -0.086, p= 0.551) and the gestational age. (r= -0.094, p= 0.514). In our study, comparing the case group by the control group (50 neonates) who were completely healthy. The study showed significant correlation between the disease and TLCs (p> 0.001), neutrophils (p >0.001), lymphocytes (p >0.001), Hb (p> 0.001), platelet count (P > 0.003) and CRP. (P> 0.001). There was a strong positive correlation between CRP and fecal calprotectin among the patients, (p = 0.045). with a significant negative correlation between platelet count and fecal calprotectin (p= 0.000).We also included that in the neonates with NEC, showed a higher level of fecal calprotectin than control. (P> 0.001).

Yoon et al. [18] determined in 2014 that there was a statistically significant difference in the CRP levels between the case and control groups. Although that difference wasn't large, still NEC group showed an increased CRP level.

Several fecal calprotectin threshold were for suspicion of NEC had been proposed, i.e, 200 mg/L in Caroll's study [19], 350 Mg/g stool in Yang study [20], 2000 Mg / g stool in Josefsson study [21], 792 Mg /g stool in Aydemir study [22] and 636 Mg /g stool for the study by Campeotto et al study [23].

Aydemir et al. [22], had a study on 25 babies having the same both the gestational age and the birth weight. The neonates whom were diagnosed having NEC showed higher fecal calprotectin levels. And the standard uptake value of the fecal calprotectin was set at 792 MG/g, the sensitivity was 76%and the specificity was 92%.

As a result, fecal calprotectin can be used as an early diagnostic marker for both pre-term and full-term infants suspected of having necrotizing enterocolitis.

CONCLUSION

We concluded that fecal calprotectin can be used as a very useful diagnostic biomarker for NEC especially in the early stage of the disease in both preterm and full term which the clinical, laboratory and radiological investigations could be non-specific.

Limitations of the study

1. High cost.
2. Time consuming.

REFERENCES

1. **Neu J and Walker WA**. Necrotizing enterocolitis. N Engl J Med. 2011;364(3):255-64.

2. **Berrington JE, Hearn RI, Bythell M, Wright C and Embleton ND**. Deaths in preterm infants: changing pathology over 2 decades. J Pediatr. 2012;160(1):49-53.e1.

3. **Zani A, Eaton S, Puri P, Rintala R, Lukac M, Bagolan P, et al.** International survey on the management of necrotizing enterocolitis. Eur J Pediatr Surg. 2015;25(1):27-33.

4. **Basu S**. Neonatal sepsis: the gut connection. Eur J Clin Microbiol Infect Dis. 2015;34(2):215-22.

5. **Samuels N, van de Graaf RA, de Jonge RCJ, Reiss IKM and Vermeulen MJ**. Risk factors for necrotizing enterocolitis in neonates: a systematic review of prognostic studies. BMC Pediatr. 2017;17(1):105.

6. **Bell MJ, Ternberg JL, Feigin RD, Keating JP, Marshall R, Barton L, et al.** Neonatal necrotizing enterocolitis. Therapeutic decisions based upon clinical staging. Ann Surg. 1978;187(1):1-7.

7. **Epelman M, Daneman A, Navarro OM, Morag I, Moore AM, Kim JH, et al.** Necrotizing enterocolitis: review of state-of-the-art imaging findings with pathologic correlation. Radiographics. 2007;27(2):285-305.

8. **Chawla S, Natarajan G, Shankaran S, Pappas A, Stoll BJ, Carlo WA, et al.** Association of Neurodevelopmental Outcomes and Neonatal Morbidities of Extremely Premature Infants With Differential Exposure to Antenatal Steroids. JAMA Pediatr. 2016;170(12):1164-72.

9. **Young C, Sharma R, Handfield M, Mai V and Neu J**. Biomarkers for infants at risk for necrotizing enterocolitis: clues to prevention? Pediatr Res. 2009;65(7):91-7.

10. **Däbritz J, Jenke A, Wirth S and Foell D**. Fecal phagocyte-specific S100A12 for diagnosing necrotizing enterocolitis. J Pediatr. 2012;161(6):1059-64.

11. **Steinbakk M, Naess-Andresen CF, Lingaas E, Dale I, Brandtzaeg P and Fagerhol MK**. Antimicrobial actions of calcium binding leucocyte L1 protein, calprotectin. Lancet. 1990;336(8718):763-5.

12. **Corbin BD, Seeley EH, Raab A, Feldmann J, Miller MR, Torres VJ, et al.** Metal chelation and inhibition of bacterial growth in tissue abscesses. Science. 2008;319(5865):962-5.

13. **Mao R, Xiao YL, Gao X, Chen BL, He Y, Yang L, et al.** Fecal calprotectin in predicting relapse of inflammatory bowel diseases: a meta-analysis of prospective studies. Inflamm Bowel Dis. 2012;18(10):1894-9.

14. **Siddiqui I, Majid H and Abid S**. Update on clinical and research application of fecal biomarkers for gastrointestinal diseases. World J Gastrointest Pharmacol Ther. 2017;8(1):39-46.

15. **Holtman GA, Lisman-van Leeuwen Y, Day AS, Fagerberg UL, Henderson P, Leach ST, et al.** Use of Laboratory Markers in Addition to Symptoms for Diagnosis of Inflammatory Bowel Disease in Children: A Meta-analysis of Individual Patient Data. JAMA Pediatr. 2017;171(10):984-91.

16. **Zhang M, Zhang X and Zhang J**. Diagnostic Value of Fecal Calprotectin in Preterm Infants with Necrotizing Enterocolitis. Clin Lab. 2016;62(5):863-9.

17. **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.

18. **Yoon JM, Park JY, Ko KO, Lim JW, Cheon EJ and Kim HJ**. Fecal calprotectin concentration in neonatal necrotizing enterocolitis. Korean J Pediatr. 2014;57(8):351-6.

19. **Carroll D, Corfield A, Spicer R and Cairns P**. Faecal calprotectin concentrations and diagnosis of necrotising enterocolitis. Lancet. 2003;361(9354):310-1.

20. **Yang Q, Smith PB, Goldberg RN and Cotten CM**. Dynamic change of fecal calprotectin in very low birth weight infants during the first month of life. Neonatology. 2008;94(4):267-71.

21. **Josefsson S, Bunn SK and Domellöf M**. Fecal calprotectin in very low birth weight infants. J Pediatr Gastroenterol Nutr. 2007;44(4):407-13.

22. **Aydemir O, Aydemir C, Sarikabadayi YU, Emre Canpolat F, Erdeve O, Biyikli Z, et al.** Fecal calprotectin levels are increased in infants with necrotizing enterocolitis. J Matern Fetal Neonatal Med. 2012;25(11):2237-41.

23. **Campeotto F, Kalach N, Lapillonne A, Butel MJ, Dupont C and Kapel N**. Time course of faecal calprotectin in preterm newborns during the first month of life. Acta Paediatr. 2007;96(10):1531-3.