Med. J. Cairo Univ., Vol. 89, No. 7, December: 2881-2892, 2021
www.medicaljournalofcairouniversity.net

Human Intestinal Fatty Acid Binding Protein as a Prognostic Marker in Premature Neonates Suffering from Necrotizing Enterocolitis

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Abstract

Background: Necrotizing enterocolitis (NEC) is a severe acute gastrointestinal disease affecting mainly preterm newborns. The pathophysiology of NEC remains poorly understood.

Aim of Study: To investigate the diagnostic and prognostic potential of this gut-associated biomarker in the early diagnostics of NEC, their association with clinically relevant and well-established disease-related parameters, and their capacity to predict the disease course among neonates in neonatal intensive care units in Ain Shams University Pediatrics Hospital.

Patients and Methods: 23 preterm neonates diagnosed with NEC were recruited randomly and enrolled in this study as the cases group, with corresponding 23 gestational age and birthweight matched preterms with no known intestinal injury or history of abdominal surgery enrolled as controls. All recruited randomly from the neonatal intensive care units of Ain Shams Pediatrics university hospital, during the period from October 2018 till March 2020.

Results: The 1st values of IFABP taken at the time of diagnosing NEC showed that mean serum IFABP concentrations of the study group were much higher than the control group. In the 2nd values of serum IFABP taken one week after diagnosing NEC showed that the mean serum IFABP concentrations of the study group became decreased in comparison with IFABP at the time of diagnosis in stages 1 and 2A compared to stages 2B and 3.

Conclusion: Serum I-FABP levels are increased in preterm neonates with NEC in comparison to age-matched controls; also serum I-FABP levels are increased according to the severity of NEC. So serial measurements of serum I-FABP levels may be a useful marker for early diagnosis and prediction of disease severity and prognosis in NEC patients.

Key Words: *Human intestinal fatty acid-binding protein – Premature neonates suffering from necrotizing enterocolitis.*

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Introduction

NECROTIZING enterocolitis (NEC) is a severe neonatal gastrointestinal disease with high morbidity and mortality (20%-40%) resulting from a combination of several factors, including genetic predisposition, intestinal immaturity, excessive intestinal inflammatory response and in appropriate microbial colonization **[1]**. Early symptoms of (NEC) are often non-specific, such as abdominal distension, bloody stools, or gastric retention **[2]**. Early identification of those patients who will eventually develop surgical NEC remains challenging, primarily because current laboratory and radiology tests lack sufficient discriminative power **[3]**. Necrotizing enterocolitis (NEC) is a severe neonatal gastrointestinal disease characterized by inflammation and intestinal cell damage **[4]** and characterized histopathologically, by intestinal coagulate or ischemic necrosis starting at the mucosa and extending into the sub mucosa and muscularis externa **[5]**.

Intestinal fatty acid binding protein (I-FABP) is a small cytoplasmic protein located in small intestinal enterocytes involved in the uptake and transport of polar lipids such as fatty acids from the small-bowel lumen **[6]**. Upon the death of the enterocyte, its cytoplasmic contents are released into the circulation, and rise in plasma I-FABP concentration has been demonstrated both in animal models and in preterm infants with NEC **[1]**. I-FABP is investigated as a measure of enterocyte damage and candidate biomarker of NEC.

Aim of the work:

The aim of this study is to determine the usefulness of serum I-FABP as a prognostic marker for prediction of severity and outcome of NEC in pre-term infants admitted in neonatal intensive care units in Ain Shams University Hospitals.

Patients and Methods

This case control study was conducted on 30 preterm neonates with suspicion of necrotizing enterocolitis (The inclusion criteria were preterm neonate aging less than 36 weeks of gestational age and not weighing more than 2500 grams at time of birth with stage IA of NEC according to modified Bell's criteria * characterized by temperature instability, lethargy, increased gastric residuals, abdominal distension, and occult blood in stool) and a control group of 23 preterm neonates without gut surgery or intestinal mucosa disruption.

All of them were recruited from the neonatal intensive care unit of Ain Shams University Pediatrics Hospital in the duration between October 2018 and March 2020.

The patients with suspected NEC were later divided into NEC group (n=23) and sepsis group (n=7) through our further follow-up and investigations (detailed perinatal and postnatal history, CBC, CRP, TLC and abdominal X-ray ± abdominal ultrasound).

The 23 neonates in the cases group 21 of them continued through out the study till the last sample collection and investigation and 2 of them died before the last sample collection.

The 23 neonates in the cases group were further divided into mild, moderate and severe cases according to their clinical presentation, hospital stay duration and the investigations done for them. Also they were classified into stages according to modified Bell's criteria.

Sample collection and processing: Two milliliters of venous blood were withdrawn under aseptic precautions and were put in suited tube (yellow cap), centrifuged and the supernatant placed in an epindorf tube at –80 Celsius degrees.

Two serial measurements were done: Once on diagnosis (8-16 hours from starting of disease progress) and another one week later.

Serum intestinal fatty acid-binding protein (IFABP): Was measured using human ELISA kits (sandwich technique). (manufactured by USA & CANADA R&D systems, Inc., and distributed by R&D China Co., Ltd.).

*Modified Bell's criteria:

- Bell's staging and suggested management for NECBell's stage	Severity	- Clinical signs and symptoms	Radiological	Treatment
I	- Mild NEC, suspected NEC	- Mild systemic signs and intestinal signs	Nonspecific	- Close clinical observation - Discontinuation of enteral feeding
II	Moderate NEC	- Moderate systemic signs with prominent abdominal distension, abdominal tenderness and wall oedema - Thrombocytopenia and metabolic acidosis	- Pneumatosis intestinalis, portal venous gas	- Medical management, such as nasograstric decompression, intravenous fluids and broad-spectrum antibiotics - Close clinical, laboratory and radiographic observation
III	Advanced NEC	- Worsening stage II signs and symptoms plus hypotension Signs of peritonitis Severe metabolic acidosis and shock	Pneumoperitoneum	- Exploratory laparotomy and resection of necrotic bowel - Peritoneal drainage in selected cases (abdominal compartment syndrome or weight <750 g)

Statistical analysis:

Data collected throughout history, basic clinical examination, laboratory investigations and outcome measures coded, entered, and analyzed using Microsoft Excel software.

The results were analyzed by using the commercially available software package (Statview, Abacus concepts, inc., Berkley, CA, USA). The parametric data were presented as mean and standard deviation (SD). In addition, non-parametric

data were presented as median and interquartile range (IQR) which is between the 25 th and 75 th percentiles. Student's *t*-test was used for comparison of parametric data, while Mann-Whitney test was used for comparison between non-parametric data. Chisquare test (χ^2) was used for comparison between qualitative variables of the studied groups. Spearman's rho correlation coefficient " *r*" was used to determine the relationship between different variables. For all tests, a probability (*p*) of less than 0.05 was considered significant and of less than 0.01 is highly significant.

Ethical consideration: Approval of the study protocol by the Ain Shams University Institutional Review Board was obtained and informed consent was obtained from the parents.

Results

The results show no significant difference between the 2 groups, which indicates that the two groups were matching and the difference in their following results isn't related to any of these factors.

This table shows a highly significant statistical difference between the two groups regarding the level of serum IFABP both at time of diagnosis of NEC and one week later.

The previous ROC curve shows that the best cut off point for IFABP score at diagnosis to differentiate between patients and controls was found >2.0ng/ml with sensitivity of 91.30%, specificity of 95.65% and area under curve (AUC) of 98.30%. While the best cut off point after 1 week was found >1.44ng/ml with sensitivity of 95.24%, specificity of 93.33% and area under curve (AUC) of 96.50%.

This table shows a highly significant statistical difference in both between the two groups owing to the disease process in the cases group.

This table shows the classification of patients into three stages:

Stage I, stage II (IIA&IIB) and stage III (IIIA &IIIB), where there are 2 patients in stage I, 15 patients in stage II (8 in stage IIA and 7 in stage IIB).

This table shows a highly significant difference between both groups where 52.2% of cases died while only 8.7% of the control group participants did.

The duration of hospital stay among patients in the cases group ranges from 3 to 60 days, with a median of 22 days.

Regarding severity, the cases were classified into mild, moderate and severe subgroups according to their clinical presentations, laboratory and radiological findings and the hospital stay duration.

In this table the univariate regression analysis shows a very high Odds ratio for the IFABP at diagnosis >2ng/ml and after 1 week >1.44ng/ml which indicates the high predictive value of this marker.

But, statistically this Odds ratio was too high to be entered for multivariate logistic regression analysis.

Also, this table shows a high odds ratio for the respiratory support (especially CPAP), thrombocytopenia and neutropenia (<2.6 10^3/ul).

A table showing highly significant correlation with the level of serum IFABP one week after NEC diagnosis.

This table shows the highly significant correlation with low platelets count (thrombocytopenia) and significant correlation with neutrophil count.

This table shows highly significant statistical difference between the two groups regarding serum IFABP level one week after NEC diagnosis.

This table shows a significant correlation with ascites and staging and highly significant correlation with resistive index in superior mesenteric artery (R.I in S.M.A) and portal venous gas.

This table shows that there is a significant difference between early stage cases and late stage cases regarding gender.

This table shows with significant difference between the two subgroups regarding IFABP level in serum at time of diagnosis of NEC and highly significant difference regarding level of IFABP in serum one week after NEC diagnosis, neutrophil count and platelet count.

This table shows univariate and multivariate regression analysis for factors associated with late staging if the disease showing the strong association between male gender and also IFABP level at diagnosis >2ng/ml with late stage disease.

Table (1): Comparison between the cases and the controls regarding gestational age, birth weight and some demographic data.

	Control group No.=23	Cases group No.=23	Test value	*p*-value	Sig.
Gestational age (weeks):					
Mean ± SD	32.87±1.60	32.26±2.12	1.100•	0.277	NS
Range	30-35	28-35			
Birth weight (kg):					
Mean ± SD	1.89±0.39	1.64±0.46	2.011•	0.050	NS
Range	1.2-2.4	0.8-2.4			
Gender:					
Female	12 (52.2%)	16 (69.6%)	1.460*	0.227	NS
Male	11 (47.8%)	7 (30.4%)			
Maternal age (years):					
Mean ± SD	28.17±4.24	27.00±5.89	0.776•	0.442	NS
Range	18-36	19-44			
Consanguinity:					
No	18 (78.30%)	18 (78.30%)	0.000*	1.000	NS
Yes	5 (21.70%)	5 (21.70%)			
Residence:					
Urban	13 (56.50%)	16 (69.60%)	0.840*	0.359	NS
Rural	10 (43.50%)	7 (30.40%)			

p-value >0.05: Non significant. *p*-value <0.05: Significant. *p*-value <0.01: Highly significant. *: Chi-square test. •: Independent *t*-test.

Table (2): Comparison between the cases group and the control group regarding the level of serum intestinal fatty acid binding protein (IFABP) at the time of diagnosis of NEC.

	Control group No.=23	Cases group No.=23	Test value#	*p*-value	Sig.
IFABP at diagnosis (ng/ml):					
Median (IQR)	0.03 (0-1.02)	13 (4-20.5)	–5.629	0.000	HS
Range	0-3	1.2-106			
IFABP after 1 week (ng\ml):					
Median (IQR)	0.06 (0-1.06)	7.5 (4-19.75)	–4.707	0.000	HS
Range	0-4.03	1.05-112			
Wilcoxon Rank test:					
Test value	1.195	0.660			
p-value	0.232 (NS)	0.509 (NS)			
Median difference:					
Median (IQR)	–0.02 (–0.45-0.1)	-2.5 (–11.95-6.0)	0.786	0.432	NS

p-value >0.05: Non significant. *p*-value <0.05: Significant. *p*-value <0.01: Highly significant. #: Mann-Whitney test.

Table (3): Comparison between cases and control groups regarding thrombocytopenia and neutrophil count.

	Control group No.=23	Cases group No.=23	Test value	*p*-value	Sig.
Platelet count (10^3/ul):					
Median (IQR)	212 (172-302)	80 (26-120)	–5.197≠	0.000	HS
Range	105-453	7-217			
Thrombocytopenia:					
Yes (present)	21 (91.3%)	4 (17.4%)	25.322*	0.000	HS
No (absent)	2 (8.7%)	19 (82.6%)			
Neutrophil (10^3/ul):					
Median (IQR)	5.2 (3.5-6.1)	2.6 (1.3-5)	–2.737≠	0.006	HS
Range	2.1-7.6	1-12.7			

p-value >0.05: Non significant. *p*-value <0.05: Significant. *p*-value <0.01: Highly significant. *: Chi-square test ≠: Mann-Whitney test.

Table (4): Staging of cases according to modified Bell's criteria.

	No. of cases in each stage	% of patients in each stage among the whole number of cases
Staging:		
I	2	8.7%
IIA	8	34.8%
IIB	7	30.4%
IIIA	5	21.7%
IIIB	1	4.3%
Radiology:		
Pneumatosis intestinalis:		
Negative	2	8.7%
Positive	21	91.3%
Increased R.I in SMA:		
Negative	10	43.5%
Positive	13	56.5%
Portal venous gas (pneumobilia):		
Negative	10	43.5%
Positive	13	56.5%
Ascitis:		
Negative	11	47.8%
Positive	12	52.2%
Intestinal perforation and pneumoperitoneum:		
Negative	22	95.7%
Positive	1	4.3%

Table (5): Comparison between cases and control groups according to the final outcome among each group (either relief or death).

	Control group No.=23	Cases group No.=23	Test value	*p*-value	Sig.
Outcome:					
Relief	21 (91.30%)	11 (47.80%)	10.268*	0.001	HS
Died	2 (8.70%)	12 (52.20%)			

p-value >0.05: Non significant.
p-value <0.05: Significant.
p-value <0.01: Highly significant.
*: Chi-square test.

Table (6): Hospital stay duration in days and severity classification among NEC patients in cases group.

Hospital stay duration (days):	
Median (IQR)	22.0 (9.0-35.0)
Range	3-60
Severity :	
Mild	2 (8.70%)
Moderate	13 (56.50%)
Severe	8 (34.80%)

Table (7): Logistic regression analysis for factors associated with the disease.

	Univariate				Multivariate			
	p-value	Odds ratio (OR)	95% C.I. for OR		*p*-value	Odds ratio (OR)	95% C.I. for OR	
			Lower	Upper			Lower	Upper
Respiratory support	0.021	4.286	1.246	14.735	0.751	1.474	0.134	16.228
CPAP	0.008	9.625	1.821	50.886	0.467	2.899	0.165	50.986
IFABP at diagnosis >2.0 (ng/ml)	0.000	231.000	19.465	2741.405				
IFABP after 1 week >1.44 (ng/ml)	0.000	280.000	16.120	4863.441				
Thrombocytopenia (10^3/ul)	0.000	49.875	8.185	303.926	0.009	17.312	2.053	146.021
Neutrophil ‹-2.6 (10^3/ul)	0.004	24.000	2.755	209.063	0.074	11.329	0.794	161.736

Table (8): Correlation between platelet count and neutrophil count with the final outcome in cases group, with a statistically significant correlation with the platelet count.

	Relief No.=11	Died No.=12	Test value	*p*-value	Sig.
Platelet count:					
Median (IQR)	105 (82-155)	32 (23.5-66.5)	–2.094≠	0.036	S
Range	7-217	9-172			
Neutrophil count:					
Median (IQR)	3.1 (2.1-5)	2.4 (1.25-4.85)	–0.586≠	0.558	NS
Range	1.2-12.7	1-6.6			

p-value >0.05: Non significant. *p*-value <0.05: Significant. *p*-value <0.01: Highly significant.
#: Mann-Whitney test. *: Chi-square test.

Table (9): Correlation between level of serum IFABP at time of NEC diagnosis and its level one week after NEC diagnosis with the disease severity among patients in cases group.

	Mild No.=2	Moderate No.=13	Severe No.=8	Test value≠	*p*-value	Sig.
IFABP at diagnosis:						
Median (IQR)	3.88 (3.25-4.5)	10 (4.74-16.5)	22.25 (10.25-40.25)	4.342	0.114	NS
Range	3.25-4.5	1.2-40	1.35-106			
IFABP After 1 week:						
Median (IQR)	4 (4-4)	4.4 (3.5-7.5)	35 (19.75-50)	10.949	0.004	HS
Range	4-4	1.05-20	10-112			
Wilcoxon Rank test:						
Test value						
p-value						
Median difference:						
Median (IQR)	0.13 (–0.5-0.75)	–6 (–11.95-0.06)	12.75 (–25-26)	1.903	0.386	NS

p-value >0.05: Non significant. *p*-value <0.05: Significant. *p*-value <0.01: Highly significant. ≠: Kruskal-wallis test.

Table (10): Correlation between platelet count (thrombo-cytopenia) and neutrophil count with disease severity among patients in cases group.

	Mild No.=2	Moderate No.=13	Severe No.=8	Test value	*p*-value	Sig.
Platelet count (10^3/ul):						
Median (IQR)	186 (155-217)	91 (52-120)	25.5 (15.5-42.5)	11.245≠	0.004	HS
Range	155-217	17-172	7-80			
Thrombocytopenia:						
No	2 (100.0%)	2 (15.4%)	0 (0.0%)	11.221*	0.004	HS
Yes	0 (0.0%)	11 (84.6%)	8 (100.0%)			
Neutrophil count (10^3/ul):						
Median (IQR)	4.5 (2.1-6.9)	3.4 (2.2-5.5)	1.25 (1.2-2.85)	6.060≠	0.048	S
Range	2.1-6.9	1.1-12.7	1-4.2			

p-value >0.05: Non significant. *p*-value <0.05: Significant. *p*-value <0.01: Highly significant. *: Chi-square test. ≠: Kruskal-wallis test.

Table (11): Comparison between mild to moderate cases and sever cases as regards level of serum IFABP at time of diagnosing NEC and its level one week after NEC diagnosis.

	Mild to Moderate No.=15	Severe No.=8	Test value≠	*p*-value	Sig.
IFABP at diagnosis (ng/dl):					
Median (IQR)	10 (3.25-16.5)	22.25 (10.25-40.25)	1.841	0.066	NS
Range	1.2-40	1.35-106			
IFABP after 1 week (ng/dl):					
Median (IQR)	4 (3.5-7.5)	35 (19.75-50)	3.283	0.001	HS
Range	1.05-20	10-112			
Wilcoxon Rank test:					
Test value					
p-value					
Median difference:					
Median (IQR)	–5 (–11.95-0.75)	12.75 (–25.0-26)	1.168	0.243	NS

p-value >0.05: Non significant. *p*-value <0.05: Significant. *p*-value <0.01: Highly significant. ≠: Mann-Whitney test.

Table (12): Correlation between level of serum IfABP one week after NEC diagnosis and other factors (thrombocytopenia, staging of cases and different radiological findings.

	IFABP after 1 week		Test value	*p*-value	Sig.
	Median (IQR)	Range			
Thrombocyopenia:					
No	4 (2.53-4.4)	1.05-4.8	–1.798≠	0.072	NS
Yes	8.5 (4-20)	2.75-112			
Staging:					
I	4 (4-4)	4-4	11.147¥	0.025	S
IIA	4.2 (3.13-6.15)	1.05-8.5			
IIB	10 (4-20)	3.5-30			
IIIA	29.88 (14.88-45)	10-50			
IIIB	112 (112-112)	112-112			
Radiology:					
Pneumatosis intestinalis:					
No	4 (4-4)	4-4	–1.083≠	0.279	NS
Yes	7.5 (4-20)	1.05-112			
Increased R.I in SMA:					
No	4 (3.5-4.8)	1.05-8.5	–2.863≠	0.004	HS
Yes	19.75 (7.5-40)	3.5-112			
Portal venous gas (pneumobilia):					
No	4 (3.5-4.8)	1.05-8.5	–2.863≠	0.004	HS
Yes	19.75 (7.5-40)	3.5-112			
Ascitis:					
No	4 (3.5-7.5)	1.05-20	–2.439≠	0.015	S
Yes	16.13 (7.5-40)	3.5-112			
Intestinal perforation and pneumoperitoneum:					
No	6.15 (4-16.13)	1.05-50	–1.658≠	0.097	NS
Yes	112 (112-112)	112-112			

p-value >0.05: Non significant. *p*-value <0.05: Significant. *p*-value <0.01: Highly significant.
≠: Mann-Whitney test. ¥: Kruskal-Wallis test.

Table (13): Comparison between the early stage cases (stage I and stage II) and late stage (stage III) cases and their correlation with different factors.

	Early stage No.=17	Late stage No.=6	Test value	*p*-value	Sig.
Gestational age (weeks):					
Mean ± SD	32.53± 1.94	31.50±2.59	1.026•	0.317	NS
Range	29-35	28-35			
Birth weight (kg):					
Mean ± SD	1.68±0.50	1.52±0.31	0.724•	0.477	NS
Range	0.8-2.4	1.2-2			
Gender:					
Female	14 (82.4%)	2 (33.3%)	5.033*	0.025	S
Male	3 (17.6%)	4 (66.7%)			
Maternal age (years):					
Mean ± SD	28.12±6.01	23.83±4.54	1.585•	0.128	NS
Range	20-44	19-32			
Consanguinity:					
No	13 (76.5%)	5 (83.3%)	0.123*	0.726	NS
Yes	4 (23.5%)	1 (16.7%)			
Residence:					
Urban	11 (64.7%)	5 (83.3%)	0.727*	0.394	NS
Rural	6 (35.3%)	1 (16.7%)			

p-value >0.05: Non significant. *p*-value <0.05: Significant. *p*-value <0.01: Highly significant.
*: Chi-square test. •: Independent *t*-test.

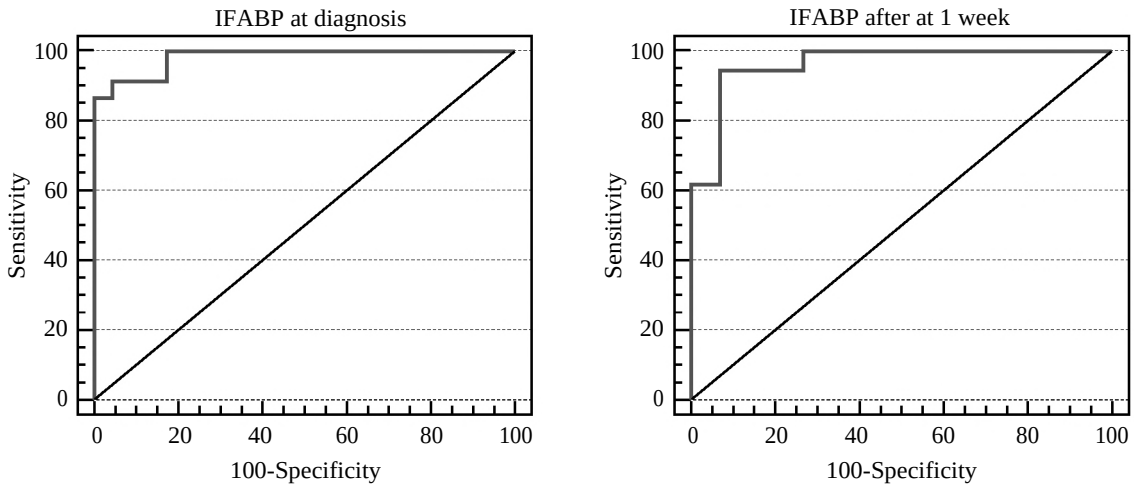
Table (14): Comparison between early stage cases and late stage cases regarding CRP, TLC, IFABP level at diagnosis of NEC, IFABP level one week after diagnosis of NEC.

	Early stage (I&II) No.= 17	Late stage (III) No.=6	Test value	*p*-value	Sig.
CRP:					
Median (IQR)	39 (11-68.6)	9.45 (6-123)	–0.771≠	0.441	NS
Range	5-300	1-250			
TLC:					
Median (IQR)	13.3 (10.2-19.3)	21.5 (13.9-44)	–1.401≠	0.161	NS
Range	6.2-140	10.2-140			
IFABP at diagnosis:					
Median (IQR)	10 (4-16.5)	29.5 (20.5-45.5)	–2.243≠	0.025	S
Range	1.2-40	1.35-106			
IFABP after 1 week:					
Median (IQR)	4.2 (3.75-8)	40 (19.75-50)	–2.901≠	0.004	HS
Range	1.05-30	10-112			
IFABP difference:					
Median (IQR)	–3.75 (–11.73-1.98)	6 (–25-19.5)	–0.495≠	0.620	NS
Range	–32.5-26	–25.75-48.65			
Hospital stay (days):					
Median (IQR)	28 (9-36)	17.5 (12-22)	–1.473≠	0.141	NS
Range	4-60	3-25			
Platelet count:					
Median (IQR)	91 (53-145)	23.5 (9-26)	–3.188≠	0.001	HS
Range	17-217	7-32			
Neutrophil:					
Median (IQR)	3.7 (2.2-5.5)	1.2 (1.2-1.3)	–3.225≠	0.001	HS
Range	1.1-12.7	1-2			

p-value >0.05: Non significant. *p*-value <0.05: Significant. *p*-value <0.01: Highly significant. *: Chi-square test. ≠: Mann-Whitney test.

Table (15): Logistic regression analysis for factors associated with late stage.

	Univariate				Multivariate			
	p-value	Odds ratio (OR)	95% C.I. for OR		*p*-value	Odds ratio (OR)	95% C.I. for OR	
			Lower	Upper			Lower	Upper
Gender (male)	0.038	9.333	1.136	76.690	0.407	3.629	0.172	76.501
IFABP at diagnosis >2 ng\ml	0.004	80.000	4.195	1525.576	0.009	54.744	2.662	1125.93



	Cut off point (ng/ml)	AUC	Sensitivity	Specificity	+PV	–PV
IFABP at diagnosis	>2.0	0.983	91.30	95.65	95.5	91.7
IFABP at 1 week	>1.44	0.965	95.24	93.33	95.2	93.3

Fig. (1): Receiver operating characteristic curve (ROC) for IFABP at diagnosis and after 1 week for differentiation between patients group and control group.

Discussion

Necrotizing Enterocolitis (NEC) is the most serious gastrointestinal disorder to occur in the premature infants. The incidence of NEC varies from 0.5 to 5 infants per 1000 live births with a mortality rate of 10 to 50% **[1,7]**.

Because NEC is characterised by loss of bowel wall integrity, intestinal fatty acid-binding protein (I-FABP) is one of the more promising biomarkers. This small cytosolic protein, located mainly in enterocytes of the small intestine, is released into the blood stream after cell disruption **[1,2,8]**.

As NEC is often a progressive disease, consecutive measurements might offer more detailed information about the disease course than a single measurement at first symptoms **[1,2,9]**.

Our aim was to determine the usefulness of serum intestinal fatty acid binding protein (I-FABP) in early diagnosis and prediction of severity of necrotizing enterocolitis (NEC) in preterm neonates admitted in Ain Shams University Hospital neonatal intensive care units.

Regarding gestational age distribution in our patients the mean gestational age was 32.26 ±2.12 weeks this is in agreement with Luig and Lui **[10]**, who confirmed that prematurity is the most important risk factor for the development of NEC because 95% of NEC cases occur in preterm infants. Premature babies are predisposed to develop NEC because of the immature mucosal barrier and immature response in addition to impaired circulatory dynamics and gastrointestinal motility. These are thought to make premature neonates at high risk for NEC higher than normal full term neonates.

Regarding Weight: In our study the weight ranged from 800 to 2400g with mean 1640 ±460g, in the study done by Aydemir et al., **[9]** they found that the mean weight was 1298 ±238g in their cases, in another study done by Guthmann et al., **[11]**.

The mean weight was 1298.7±572.5g in neonates who developed NEC.

Regarding sex 7 were males (30.4%) and 16 were females (69.6%) with a male to female ratio 1:2. In contrast to our results, McGuire et al., **[12]** reported that male gender is one of perinatal risk factors for NEC.

In our study clinical presentation of NEC shows signs of feeding intolerance (abdominal distension with gastric residuals or occult blood in stools) and neurological signs (lethargy and weak reflexes). This was in agreement with the study by Bush et al., **[12]**.

CBC was done to all cases and showed thrombocytopenia in cases group and normal platelet count in control group with a significant statistical decrease in cases group. This was in agreement with the previous study done by Hallstrom et al., **[3]** who reported that thrombocytopenia is a common laboratory finding in patients with proven NEC.

In our study, CRP was positive in 95% of cases of case group in agreement with Abdel-Haie et al. **[1]** where CRP was positive in 83% of cases.

Total leucocytic count ranged from 6.2 (10 ^3/ul) to 140 (10^3/ul) in the cases group with a median of 14.2 (10^3/ul).

In a recent study, Lambert et al., **[32]** noted that infants who died of fulminant NEC within 48 hours of onset may have low blood lymphocyte counts. They reviewed the medical records of 523 infants with a diagnosis of NEC, of which 35 (6.7%) had a fulminant course. These infants were more likely to have a blood lymphocyte count <4000/ L (*p*=0.018), besides having portal venous air, severe anemia, recent increase in feeding volume or introduction of human milk fortifier, and increased immature to total neutrophil ratio.

Neutrophil count ranged from 1 to 12.7 (10^3/ul) in the same group.

Increased neutrophil counts comprise an appropriate inflammatory response in patients with mild-moderately severe disease. In contrast, neutropenia, defined as an absolute neutrophil count less than 1500/ L, can be seen in severe NEC and is associated with adverse outcome. Patel et al., **[13]** noted that 14 of their 23 patients who died of NEC were neutropenic, compared to 6/24 of the survivors. In another study, Ragazzi et al., **[14]** noted that the detection of neutropenia in the initial blood counts at the time of onset of NEC was associated with adverse outcome; there was a trend for a higher frequency of neutropenia in non-survivors (37%) than in survivors (25%; *p*=0.136), and thrombocytopenia and neutropenia occurred together more frequently in non-survivors (39%) than in survivors (14%; *p*=0.0007).

In our study, regarding serum IFABP values; the 1 st values of IFABP at time of diagnosing NEC showed that the range of serum IFABP concentrations in the cases group is 1.2-106 (ng/ml) with a

median of 13 (ng/ml), and that of the control group were 0-3 (ng/ml) with a median of 0.03 (ng/ml).

The 2nd values of serum IFABP taken one week after diagnosing NEC showed that the mean serum IFABP concentrations of the study group were 1.05 to 112 (ng/ml) with a median of 7.5 (ng/ml) which became decreased in comparison with IFABP at the time of diagnosis regarding stages 1 and 2A while still higher increasing levels noticed in stage 2B and 3. Thus meaning it could distinguish medically treatable cases from more severe cases that may need exta intervention as ventilator support and surgical management. With levels in control group ranging from0 to 4.03 (ng/ml).

This was in agreement with previous studies by Abdel-Haie et al., **[1]** and El Bana et al., **[2]** who found that serum I-FABP taken 7e10 days after NEC, at the last day of antibiotic treatment with the continuous sampling showed that serum I-FABP levels decrease during the therapy.

Similar to our results, they found significantly higher I-FABP levels in NEC infants than in the control group. Recently, in a study comparing I-FABP with other potential markers, Benkoe et al., [15]reported that I-FABP concentrations were significantly higher in NEC infants compared with controls.

Our results demonstrated that I-FABP had diagnostic and prognostic properties. This was in agreement with the previous study by Ng et al., [16]and Ehab El Bana et al., **[2]** who showed that I-FABP is a potentially useful biomarker for differentiating between NEC and control patients with suspected clinical sepsis or NEC.

Regarding serum IFABP according to Bell's staging we found significantly higher levels of serum I-FABP in infants with stages 2 and 3 NEC compared to stage 1 NEC with a sensitivity of 91.30% and a specificity of 95.65%. in agreement with Abdel-Haie et al., **[1]** and El Banna et al., **[2]**.

Furthermore we demonstrated that significantly higher I-FABP values 1 week after disease are related to the severity of NEC. Higher serum I-FABP levels 1 week after NEC were found in infants with stage 3 NEC compared with stage 1 or 2 NEC. Serum I-FABP level was gradually decreased from the onset of the disease to 1 week in stage 1 and stage 2A NEC and slightly decreased in stages 2B and 3.

We plotted ROC curve for serum I-FABP as a diagnostic and prognostic marker and it showed 91.30% sensitivity and 95.65% specificity at time of diagnosis and 95.24% sensitivity and 93.33% specificity one week later.

Also, plotting ROC curve for serum I-FABP in the detection of NEC stage which revealed a cutoff value of serum I-FABP 2.0 (ng/ml) at diagnosis of NEC.

In agreement with Schurink et al., **[17]** who demonstrated that as early as the first eight hours after the onset of non-specific gastrointestinal symptoms, I-FABP had valuable diagnostic and prognostic properties.

Abdel-Haie et al., **[1]** reported that the 1st values of IFABP taken at birth showed that mean serum IFABP concentrations of the study group were higher than that of the control group. And IFABP taken at the time of diagnosing NEC showed that mean serum IFABP concentrations of the study group were higher than the control group.

Thuijls et al., **[18]** concluded, based on single samples, that I-FABP could improve early diagnosis of intestinal ischemia. In the same year, a study on I-FABP measured in serum instead of plasma, Terrin et al., **[19]** reported that intestinal fatty acid-binding protein with senitivity100% and specificity 91% when tested to predict the evolution from definite to advanced NEC.

Conclusion:

Serum I-FABP levels are increased in preterm neonates with NEC in comparison to age-matched controls; also serum I-FABP levels are increased according to the severity of NEC. So serial measurements of serum I-FABP levels may be a useful marker for early diagnosis and prediction of disease severity and prognosis in NEC patients.

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البروتين البشرى الرابط للأحماض الدهنية المعوية وأهميته كدلالة للتنبؤ بنتائج الشفاء لدى الأطفال المبتسرين الذين يعانون من إلتهاب الأمعاء الناخر

المقدمة: يعتبر إلتهاب الأمعاء والقولون الناخر أحد الأمراض المعوية الحادة ويصيب بشكل رئيسى الأطفال حديثى الولادة المبتسرين. وتظل الفسيولوجيا المرضية لهذا المرض غير مفهو مة جيداً.

الهدف من هذه الدراسة: بحث الاحتمالات التشخيصية والتنبؤية لهذه االعلامة الحيوية المرتبط بالأمعاء فى التشخيص المبكر لإلتهاب الأمعاء والقولون الناخر وارتباطها بالمعايير ذات الصلة سريرياً والراسخة ذات الصلة بالأمراض، وقدرتها على التنبؤ بمسار المرض بين الولدان فى وحدات العناية المركزة لحديثى الولادة بمستشفى الأطفال بجامعة عين شمس.

المرضى وطرق البحث: شملت الدراسة ٢٣ من الأطفال حديثى الولادة المبتسرين الذين تم تشخيص إصابتهم بهذا المرض وبشكل عشوائى وتم تسجيلهم فى هذ الدراسة كمجموعة الحالات، بالإضافة إلى عدد ٢٣ من الأطفال حديثى الولادة المتوافقين فى عمر الحمل والوزن عند الولادة مع عدم وجود إصابة معوية معروفة أو تاريخ من جراحة البطن مسجلين كعناصر تحكم. وحدات مستشفى جامعة عين شمس للأطفال، خلال الفترة من أكتوبر ٢٠١٨ حتى مارس ٢٠٢٠. خضع جميع المشاركين للفحص السريرى الكامل، والأشعة السينية للبطن وقياس مصل البروتين الرابط للأحماض الدهنية بالأمعاء.

النتائج: أظهرت القيم الأولى لمتوسط قياس البروتين المأخوذة فى أول وقت تشخيص المرض لدى مجموعة الحالات ارتفاعاً واضحاً أعلى من متوسط مستوى متوسط قياس البروتين عند المجموعة الضابطة. وفى القيم الثانية لمتوسط مستوى البروتين فى بلازما الدم لدى الحالات كان المتوسط أعلى أيضاً فى الحالات من المجموعة الضابطة ولكنه أقل ما بين مجموعة الحالات المصنفة بالمرحلة الأولى من المرض ١، وكذلك المرحلة الثانية أ من متوسط الامستوى الأول عند و قت التشخيص فى حين كان مازال الارتفا ع مستمراً وفى ازدياد واضح فى متوسط مستوى البروتين لدى الحالات الأكثر خطورة فى المرحلة الثانية ب والمرحلة الثالثة.

الخلاصة والاستنتاجات: قد تكون القياسات التسلسلية لمستويات مصل الدم للبروتين الرابط للأحماض الدهنية بالأمعاء علامة حيوية مفيدة للتشخيص المبكر والتنبؤ بخطورة المرض فى مرض إلتهاب الأمعاء والقولون الناخر.