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Serum Intestinal Fatty Acid Binding Protein Level in Diagnosis of Preterm Neonates with Necrotizing Enterocolitis

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ABSTRACT

Background: The intestinal villi's tip, which is frequently wounded in necrotizing enterocolitis (NEC), contains cells that contain intestinal fatty acid binding protein (I-FABP). In this study, we looked at the use of serum I-FABP in predicting the severity and early diagnosis of NEC. Early signs of NEC, such as abdominal distension, bloody stools, or gastric retention, are sometimes vague. Intestinal coagulate or ischemia necrosis that begins at the mucosa and spreads into the submucosa and muscularis externa are histological characteristics of NEC.

Objectives: To assess the amount of intestinal fatty acid binding protein in the blood as a possible biomarker for early diagnosis and prognosis in preterm neonates with necrotizing enterocolitis.

Patients and methods: This is a prospective cohort study conducted on 40 pre-term neonates of both sexes in NICU at Menoufia University Hospital, during the period from March 2019 till April 2022.

Results: Capillary refilling time was significantly prolonged, while RR and HR were lower in patients than in controls. Hb, PLT counts and serum levels of Na, K and Ca were significantly lowered among patients than controls. C-reactive protein was significantly increased among patients' group (4.45 ± 1.01) than controls group (1.35 ± 0.32), (P= 0.001). Also, 16 (80%) in patients with NEC had no growth in blood culture with a significant difference (P= 0.035) and mean serum intestinal fatty acid binding protein level was significantly higher at diagnosis in patients group (73.3 ± 11.1) than in control group (4.87 ± 1.11),(P= 0.001). ROC curve analysis showed that cutoff point of serum intestinal fatty acid binding protein in diagnosis of preterm neonates with necrotizing enterocolitis was 7.75 ng/ml with sensitivity of 100%, and specificity of 100% at AUC of 1.00 and bell stage in necrotizing enterocolitis was 72.5 ng/ml with sensitivity of 86%, and specificity of 100% at AUC of 1.00.

Conclusion: According to the clinical presentation of NEC, serum I-FABP levels were higher in preterm neonates with NEC compared to age-sex matched controls. Serum I-FABP levels were also higher according to the severity of NEC. Serial assessments of serum I-FABP levels might therefore be a helpful indicator of NEC prognosis. Serum I-FABP can therefore be a reliable serologic biomarker for the early diagnosis, prognosis, and assessment of the severity of NEC in preterm neonates.

Keywords: Necrotizing enterocolitis, Preterm neonates, Serum intestinal fatty acid.

INTRODUCTION

One of the most frequent gastrointestinal problems in preterm neonates is necrotizing enterocolitis (NEC). Ischemic necrosis of the intestinal mucosa, acute inflammation, enteric gas-forming organism invasion, and gas dissection into the intestinal wall and portal venous system are the main symptoms of this condition [1]. Short bowel syndrome, abnormal growth, and cognitive delay are among the important long-term morbidities seen by neonatal intensive care survivors, particularly preterm very low birth weight (VLBW) infants (BW 1500 g) [4]. NEC is caused by a number of factors, including genetic predisposition, intestinal immaturity, excessive intestinal inflammatory response, and inappropriate microbial colonisation [3].

After enteral feedings begin in the second week of life, NEC frequently shows up. The outcomes of the physical examination, laboratory testing, and abdominal radiographs are used to make the diagnosis of NEC. Neonatal patients with necrotizing enterocolitis have regular abdominal exams and radiographs done, and they may require surgery or primary peritoneal drainage for perforation or necrosis [4]. Current laboratory and radiological diagnostics don't have enough discriminative strength, which makes it difficult to identify people early who will later have definitive NEC [5]. I-FABP is one of the more promising biomarkers for NEC since it is associated with loss of intestinal wall integrity. After cell breakdown, this little cytosolic protein, which is mostly present in small intestine enterocytes, is released into the bloodstream [6]. Within hours of tissue injury, IFABP is easily eliminated by the kidneys and may be detected in both plasma and urine [7, 8]. In babies who have abdominal symptoms, IFABP may be utilised to make an early diagnosis and determine the severity of NEC [9].

The purpose of this study was to assess the amount of intestinal fatty acid binding protein in the blood of preterm neonates with necrotizing enterocolitis in relation to clinical symptoms as a possible diagnostic and prognostic biomarker.

PATIENTS AND METHOD

This is a prospective cohort study that was conducted on 40 pre-term neonates of both sexes in NICU at Menoufia University Hospital, during the period from March 2019 till April 2022.

Included neonates were classified into two groups: Patient group that included 20 preterm neonates diagnosed with NEC according to Modified Bell's

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Staging criteria of NEC [10] and control group, which included 20 clinically healthy preterm neonates of matching gestational age, birth weight and sex admitted to NICU.

Inclusion criteria: Preterm neonates with gestational age less than 37 weeks, of both sexes who met the diagnostic modified Bell's staging criteria of NEC.

Exclusion criteria: Full term neonates, congenital anomalies, inborn errors of metabolism, fatal chromosomal defects, liver and kidney dysfunctions, surgical disorders, intestinal diseases and need for exchange transfusion during the period of study.

All patients and control neonates were subjected to the following:

- **Detailed history taking:** Prenatal history such as maternal diseases (diabetes mellitus, hypertension), premature rupture of membranes, uterine rupture, or placenta and umbilical cord disorders and infection. Natal history, mode of delivery and postnatal history. Family history (Consanguinity or previous similar conditions).
- **Clinical examination:** Abdominal examination and bowel movement and related findings (vomiting, abdominal distension, tenderness, and heme-positive stool). Modified Bell's criteria (Classifying the degree of NEC: Grades1, 2, and 3 correlate with the description of suspected mild, moderate, and severe NEC) [10].
- **Laboratory & Radiological Investigations:** Serial abdominal plain X-ray films for patients' group and once for control group. Imaging findings were dilated loops, pneumatosis intestinalis, ascites and pneumoperitoneum and bowel perforation.

Laboratory investigations:

Sampling: Samples of blood were withdrawn under aseptic precautions and were put in EDTA vacutainer (violet cap) and mixed up & down gently, then used for measuring Complete blood count (CBC). Samples of blood were put in plain test tubes without anticoagulant. After coagulation, they were centrifuged (at 2000 r.p.m. for 20 minutes), then supernatant was removed to test for C-reactive protein (CRP), serum electrolytes (Na, K, Ca) and specific investigations including serum level of I-FABP by ELISA Reader (Double antibody sandwich Elisa, normal value: 2.0 ng/mL or less). Arterial or venous blood gases (A or VBG) were done for evidence of acidosis, hypoxia or hypercarbia.

Ethical approval: Menoufia University's ethics committee gave its approval for this work. Written informed consents were obtained from parents before they agreed to participate in the study. The Helsinki Declaration was followed throughout the study's conduct.

Statistical Analysis

To conduct the quantitative study, we used version 24 of the Statistical Package for the Social Sciences (SPSS). Tables and graphs were used to display the information. The numerical data was presented with their respective means, medians, standard deviations, and confidence intervals. Data visualisations made use of numerical examples, such as frequency and %. The student's t test (T) was frequently used for analysing quantitative data with independent variables. Using Pearson's Chi-Square and Chi-Square for Linear Trend, we analysed data that was qualitatively different from one another (X^2). Hosmer and Lemeshow test was used to detect goodness of fitness of logistic regression model. To be statistically significant, we determined that a P value of 0.05 or lower was necessary.

RESULTS

There were no discernible differences between the groups of patients and controls concerning gestational age, age of onset, sex, mode of delivery (P>0.05), while capillary refilling time was significantly prolonged among patients (5.75 ± 0.71 sec) than in controls (2.10 ± 0.64 sec), (P=0.001). Respiratory rate and heart rate were significantly lowered among patients than in controls (P= 0.001). Contrarily, there were no appreciable variations in weight, length, head size, chest circumference, or temperature between patients and controls (P>0.05) (Table 1).

Table (1): Demographic and clinical characteristics of the two studied groups (N=40)

Studied variables	The studied groups		Difference (%)	t	P value
	Patients (N=20)	Controls (N=20)			
Gestational age/ weeks: Mean ± SD Median Range	28.9±1.48 29.0 (27-32)	28.5±1.31 28.0 (27-31)	1.404%	0.789	0.435
Age of onset/ days: Mean ± SD Median Range	15.6±6.82 14.5 (6-30)	15.7±6.73 14.5 (6-30)	0.637%	0.047	0.963
Sex:	**N (%)**	**N (%)**	10.000%	X^2= 0.100	0.752
Male Female	9 (45.0) 11 (55.0)	10 (50.0) 10 (50.0)			
Mode of delivery: Vaginal Caesarian	5(25.0) 15(75.0)	9(45.0) 11(55.0)	20.000%	1.75	0.185
Weight/ gram at diagnosis: Mean ± SD	1017.1±172.8	1026.7±173.3	0.944%	0.177	0.860
Length /cm: Mean ±SD	37.6±3.88	38.1±3.21	1.312%	0.444	0.660
Head circumference (cm): Mean ± SD	27.2±2.21	27.7±1.86	1.805%	0.850	0.400
Chest circumference (cm): Mean ± SD	25.2±2.19	24.6±2.06	2.439%	0.892	0.378
CRT/ sec: Mean ± SD	5.75±0.71	2.10±0.64	173.810%	5.45	**0.001****
Temperature/ ºC: Mean ± SD	37.0±0.32	37.1±0.10	0.270%	1.58	0.113
Respiratory rate /min: Mean ± SD	37.2±3.55	42.5±2.23	12.470%	5.59	**0.001****
Heart rate/min: Mean ± SD	88.6±7.35	130.5±11.6	32.107%	13.5	**0.001****

t: Independent t-test. X^2: Chi squared test. **High significant. **CRT:** Capillary refill time.

Hemoglobin and platelets count were significantly lowered among patients than in controls (P<0.05). While there were no significant differences regarding white blood cells count (P>0.05). Serum levels of sodium, potassium, and calcium were significantly lowered among patients' group than in controls (P<0.05) (Table 2).

Table (2): CBC and Electrolytes among patients and controls studied groups (N=40)

Studied variables	The studied groups		Difference (%)	t	P value
	Patients (N=20)	Controls (N=20)			
CBC					
Hemoglobin(gm\dl): Mean ±SD	10.2±1.59	16.5±1.81	61.77%	11.6	**0.001****
WBCs (mm^3): Mean ± SD	13.1±3.12	10.7±1.53	23.53%	1.71	0.086
Platelet(mm^3): Mean ±SD	59.4±13.7	318.6±69.4	100.00%	5.41	**0.001****
Electrolytes					
Na (mEq\L): Mean ± SD	125.3±4.29	139.0±3.03	10.94%	11.7	**0.001****
K(mEq\L): Mean ±SD	3.00±0.63	3.91±0.30	30.33%	5.76	**0.001****
Ca(mg\L): Mean ±SD	8.72±0.84	9.28±0.61	6.42%	2.42	**0.020***

WBCs: White blood cells. **X^2:** Chi squared test. ****High significant. **Na:** Sodium. **K:** Potassium. **Ca:** Calcium. **t**: Independent t-test. **X^2:** Chi squared test. ****High significant.

C-reactive protein was significantly increased among patients (4.45 ± 1.01 mg\L) than in controls (1.35 ± 0.32 mg\L), (P= 0.001). Positive blood culture was found in 20% of patients (P= 0.035). As regards serum level of intestinal fatty acid binding protein, it was significantly higher at diagnosis in patients' group (73.3 ± 11.1 ng/ml) than in controls group (4.87 ± 1.11 ng/ml), (P= 0.001) (Table 3).

Table (3): C-reactive protein, blood culture and serum intestinal fatty acid binding protein level at **diagnosis** among patients and controls studied groups (N=40).

Studied variables	The studied groups		Difference (%)	t	P value
	Patients (N=20)	Controls (N=20)			
CRP (mg\L): Mean ±SD	4.45±1.01	1.35±0.32	69.663%	3.67	**0.001****
Blood culture	N (%)	N (%)	20.000%	X^2= 4.44	**0.035***
Positive	4(20.0)	0(0.00)			
Negative	16(80.0)	20(100)			
Serum intestinal fatty acid binding protein level at diagnosis (ng/ml): Mean ±SD	73.3±11.1	4.87±1.11	_	5.42	**0.001****

CRP: C-reactive protein. **t**: Independent t-test. **X^2:** Chi squared test. ******High significant. **t**: Independent t-test. ******High significant. NB: Normal level of CRP<6 mg/l

In addition, mean serum intestinal fatty acid binding protein level was significantly decreased after one week in patients with treatment (16.3 ± 3.90 ng/ml) than at diagnosis (73.3 ± 11.1 ng/ml), (P= 0.001), **(Table 4).**

Table (4): Comparison between serum intestinal fatty acid binding protein level at diagnosis and after one week among the studied patients (N=20)

Studied variable	The studied cases		Paired t-test	P value
	At diagnosis	After 1 week		
Serum intestinal fatty acid binding protein level (ng/ml) Mean ±SD	73.3±11.1	16.3±3.90	19.6	**0.001****

t: Independent t-test. ******High significant.

Mean serum intestinal fatty acid binding protein level at diagnosis was significantly higher among patients with stage III than patients with stage II, that was significantly higher than stage I, (87.5 ± 4.98, 73.5 ± 3.72 & 60.8 ± 4.91 ng/ml respectively, P= 0.001 ng/ml). It was significantly increased among patients with 3B Bell stage (93.5 ± 0.70 ng/ml) compared to other Bell stages, with a significant difference (P=0.003). There were no significant differences between serum intestinal fatty acid binding protein levels after 1 week and bell stages of the studied patients (P=0.289). However, it was significantly higher among patients with stage III (18.7±4.08 ng/ml) than patients with stage II (13.5 ±3.27 ng/ml), with p value 0.007. Serum level of intestinal fatty acid binding protein after one week was significantly increased among patients with Bell stage (1A), (25.0±0.00 ng/ml) compared to other Bell stages, with P=0.035. Difference in subgroups were higher in stage III than in stage II than in stage I, especially stage B more than stage A. More ever, there was significant decreased improvement among serum intestinal fatty acid binding protein level after 1 week than serum intestinal fatty acid binding protein level regarding at diagnosis stage II with %improvement (-444.4%) with mean change (-60±1 ng/ml) (Table 5).

Table (5): Relation between serum level of intestinal fatty acid binding protein level at diagnosis, after one week and bell stage of the studied patients at diagnosis and after 1 week (N=20)

At diagnosis		Bell stage			F	P value
		Stage I	Stage II	Stage III		
Serum intestinal fatty acid binding protein level at diagnosis (ng\ml)	Mean ±SD	60.8±4.91	73.5±3.72	87.5±4.98	52.5	**0.001****
Post hoc		**P1=0.004**, P2<0.001**, P3:0.001****				
After one week		**Stage I**	**Stage II**	**Stage III**		
Serum intestinal fatty acid binding protein level after one week (ng\ml)	Mean ±SD	16.7 ±4.16	13.5 ±3.27	18.7±4.08	1.35	0.289
Mean changes		-44.1±2.26	-60.00±1.00	68.8±0.9		
% Improvement		-264.07%	-444.4%	-367.91%		

At diagnosis		Bell stage						Kruskal Wallis test	P value
		1A	1B	2A	2B	3A	3B		
Serum intestinal fatty acid binding protein level at diagnosis (ng\ml)	Mean ±SD	55.0± 0.00	63.3± 2.88	67.7± 2.51	75.7± 1.50	85.5 ± 3.93	93.5 ± 0.70	18.1	0.003**

After one week		Bell stage					Kruskal Wallis test	P value
		1A	1B	2A	2B	3A		
Serum intestinal fatty acid binding protein level after one week (ng\ml)	Mean ±SD	25.0± 0.00	13.6± 3.30	9.66± 0.57	15.2± 3.64	18.7± 4.08	10.3	**0.035***
Mean changes		30.0± 00.0	-49.7± 1.13	58.04± 1.94	-60.5± 3.49	-66.8±3.38		
% Improvement		-120%	-365.4%	-600.8%	-398.02%	-357.2%		

F: ANOVA F test. ******High significant. **P1:** Stage 1 compared stage 2. **P2:** Stage 1 compared stage 3. **P3:** Stage 2 compared stage 3.

ROC curve analysis showed that cutoff point of serum level of intestinal fatty acid binding protein in diagnosis of preterm neonates with necrotizing enterocolitis was 7.75 ng/ml with sensitivity of 100%, and specificity of 100% at AUC of 1.00 and bell stage in necrotizing enterocolitis was 72.5 ng/ml with sensitivity of 86%, and specificity of 100% at AUC of 1.00. (Table 6).

Table (6): Roc curve for sensitivity and specificity of serum intestinal fatty acid binding protein in diagnosis of preterm neonates with necrotizing enterocolitis and detection of bell stage in necrotizing enterocolitis.

Variable	AUC	P value	Cutoff point	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)
Serum intestinal fatty acid binding protein	1.00	**0.001***	7.75	100%	100%	100%	100%	100%
Serum intestinal fatty acid binding protein	1.00	**0.001***	72.5	86%	100%	100%	75%	90%

*****Significant. **AUC:** Area under curve **PPV:** Positive predictive value **NPV:** Negative predictive value.

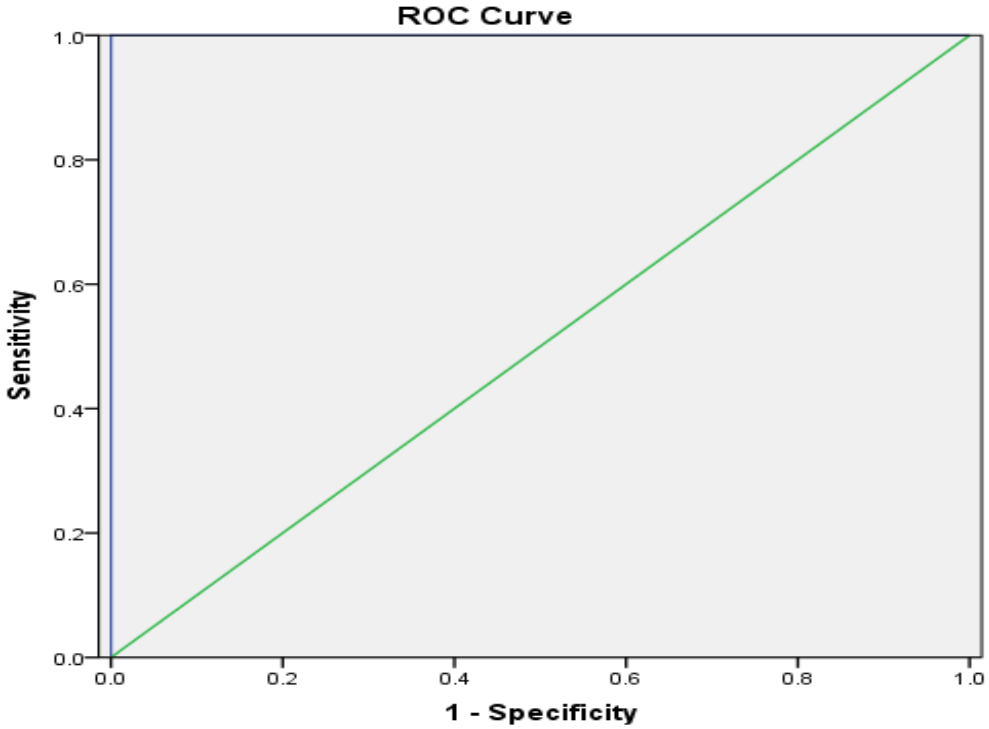

Figure (1): Roc curve for sensitivity and specificity of serum level of intestinal fatty acid binding protein at diagnosis of preterm neonates with necrotizing enterocolitis.

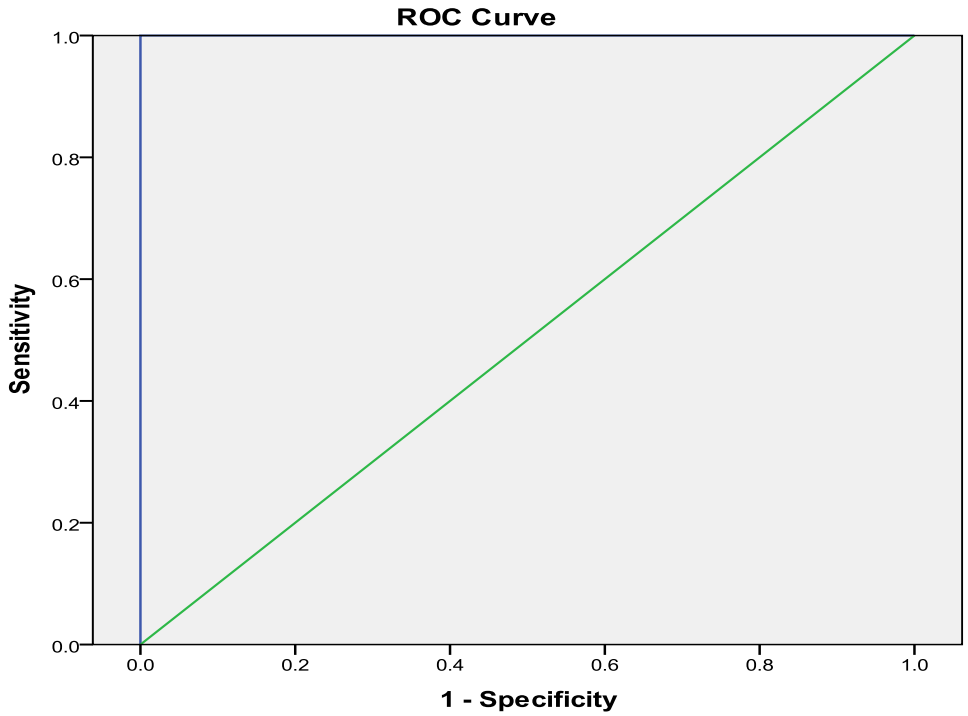

Figure (2): Roc curve for sensitivity and specificity of serum level of intestinal fatty acid binding protein at diagnosis in detection of bell stage III in necrotizing enterocolitis.

DISCUSSION

The gastrointestinal tract is inflamed and necrosed in the deadly newborn illness known as necrotizing enterocolitis (NEC). Though it is associated with reduced intestinal wall perfusion and ischemia, particularly in juvenile guts with disruption of the intestinal barrier, activation of inflammatory mediators, and permitting of bacterial passage [11], its aetiology is still not fully understood. The intracellular protein known as FABP is tiny (14–15 kDa), rises in inflammatory and ischemia-related situations, and is crucial for defending cells against the negative effects of fatty acids [12]. I-FABP is one of the most optimistic indicators since it reflects the loss of gut wall integrity that characterises NEC. This little cytosolic protein, which is mostly present in enterocytes in the small intestine, is released into the bloodstream during cell disintegration [13].

There were no discernible differences between patients and controls in terms of gestational age, age of onset in patients, sex, weight, or method of delivery in a total of 40 preterm neonates (P>0.05). In a similar vein, **Abdel-Haie *et al.*** [13] discovered that the mean gestational age was 32.3 ± 1.8 weeks in their patients with reference to gestational age dispersion. This concurs with the findings of **Luig *et al.*** [14] who established that prematurity is the main risk factor for the emergence of NEC because 95% of NEC cases involve preterm neonates. Due to the juvenile mucosal barrier, immature response, as well as compromised circulatory dynamics and gastrointestinal motility, premature neonates are more likely to develop NEC. When compared to typical full-term babies, these are thought to raise the risk of NEC in preterm neonates.

According to our study, the mean weight of the patients was 1017.1 ± 172.8 g. Additionally, concerning sex, a male to female ratio of 1:1, with 9 men (50.0%) and 9 females (50%) in total according to **Abdel-Haie *et al.*** [13]. While in our survey, 11 (55.0) females were the most prevalent, compared to 9 (45.0) men. The weight at diagnosis varied from 1000 to 2000 g, with a mean of 1451.82 ± 76.7 g,**.**

Haemoglobin and platelet levels were substantially lower in patients than in controls, according to the current study (P 0.05). Regarding white blood cells, there was no discernible difference between patients and controls (P>0.05). Our findings concur with those of the research by **Abdel-Haie *et al.*** [13]**,** who noted that the CBC revealed thrombocytopenia in the NEC group (group I) and a normal platelet count in the group II, with a substantial statistical drop in the NEC group (group I). Anaemia was another laboratory result in our investigation; patients' median haemoglobin levels were 10.35 ± 1.28g/dl, compared to controls' mean haemoglobin levels of 16.5 ± 1.81g/dl. Additionally, **Hallstrom *et al.*** [5] noted in a prior research that thrombocytopenia is a frequent laboratory finding in individuals with established NEC. In order to aid a seamless transition from the aquatic uterine environment, it is essential to have accurate information of the physiological changes in body water and solute after birth, according to **Chawla *et al.*** [15]. Furthermore, they found that abnormalities of fluid and electrolyte are prevalent in neonates. The newborn kidney's ability to eliminate extra water and salt is restricted, and an excess of either during the first week of life can lead to morbidities such as chronic lung disease, patent ductus arteriosus, and necrotizing enterocolitis.

In our study, patients had considerably higher mean serum intestinal fatty acid binding protein levels at diagnosis (73.31 ± 1.1 ng/ml) than controls (4.87 ±1 .11 ng/ml), (P=0.001). This is in line with the findings of **Coufal *et al.*** [16] who discovered that I-FABP levels were noticeably greater in patients with NEC than in controls. Additionally, our investigations revealed that the cases group had considerably higher levels of C-reactive protein (4.45 ± 1.01 ng/ml) than the controls group (1.35 ± 0.32 ng/ml), with a P value of 0.001. As a result of this worry, **Philip *et al.*** [17] observed elevated CRP in 12 (80%) of 15 children with classical NEC at initial examination, although they did not specify the precise time gap between CRP measurement and the beginning of clinical symptoms.

The results of the current investigation demonstrated that the mean serum intestinal fatty acid binding protein level at diagnosis was substantially higher in stage III patients (87.5 ± 4.98 ng/ml) than in stage II patients (73.5 ± 3.72 ng/ml), and it was also significantly higher than in stage I patients (60.8 ± 4.91 ng/ml), with a P value of 0.001. In the first quantitative radioimmunoassay for human I-FABP, it was discovered that I-FABP levels were greater in NEC patients. Ischemia and loss of intestinal wall integrity are two variables in the pathogenesis of NEC. Plasma I-FABP can therefore be utilised as a diagnostic indicator for early intestinal mucosal deterioration [18]. **Abdel-Haie *et al.*** [13] found that the mean serum IFABP in stage 1 was 115.71 ± 35.99 ng/ml, the mean serum IFABP in stage 2 was 290.16 ± 90.49 ng/ml, and the mean serum IFABP in stage 3 was 641.86 ± 19.02 ng/ml based on Bell's staging at the time of NEC diagnosis. This is consistent with the results of the study by **Aydemir *et al.*** [19]**,** which revealed that the mean serum I-FABP concentrations of stage 1, stage 2, and stage 3 at 24 hours of NEC were 112.5 ± 84.2 ng/ml, 269.4 ± 269.8 ng/ml, and 317.4 ± 365.3 ng/ml, respectively. Additionally, **Edelson *et al.*** [20] discovered that higher I-FABP was observed in the blood of all neonates with stage 3 NEC and 3 of 24 infants with stage 1 or stage 2 NEC. Another study looking at the value of monitoring plasma I-FABP levels for NEC diagnosis found that neonates with NEC had higher plasma I-FABP levels than healthy controls. Additionally, their research

revealed that more severe NEC was associated with greater I-FABP plasma levels [21].

Our research found no association between Bell phases of the patients under study and blood intestinal fatty acid binding protein levels after one week (P=0.289). However, there was a substantially greater among stage III patients (18.7 ± 4.08 ng/ml) compared to stage II patients (13.5 ± 3.27 ng/ml), with a p value of 0.007. Additionally, **Abdel-Haie *et al.* [13]** showed a correlation between the severity of NEC and considerably increased I-FABP readings 1 week after the sickness. Babies with stage 3 NEC had higher serum I-FABP levels than babies with stage 1 or stage 2 NEC one week following NEC. In stage 1 and stage 2 NEC, the serum I-FABP level steadily dropped from the time of the disease's inception to one week, and it barely changed in stage 3.

In our study, the mean serum intestinal fatty acid binding protein level was shown to be considerably lower in patients after one week (16.3 ± 3.90 ng/ml) compared to upon diagnosis (73.31 ± 1.1 ng/ml), with a significant drop (P=0.001). The mean 72nd hour IFABP value was considerably higher in operated babies compared to other neonates at diagnosis according to a previous research by **Aydemir *et al.* [19].**

At the time of diagnosis, there were substantial negative relationships between the blood levels of intestinal fatty acid binding protein and haemoglobin, platelet count, sodium, potassium, and calcium (P>0.05). Association between plasma and urine I-FABP levels and resected bowel length was shown in another investigation by **Heida *et al.* [22]**. Additionally, they demonstrated a substantial correlation between urine I-FABP levels and colon resection length. In a previous study, **Schurink *et al.* [23]** found that I-FABP in urine is not only a good biomarker for the presence of NEC but may also be highly helpful in separating instances of surgically treated NEC from patients treated conservatively. These findings are significant because they support our theory that I-FABP levels are a marker of enterocyte damage during NEC development.

The terminal ileum is frequently affected, despite the fact that the pattern of intestinal involvement in NEC can be fairly varied. The jejunum exhibits the highest tissue concentration of I-FABP expression across the intestinal tissue. Although neither the detection of plasma levels of I-FABP nor the fusion of various fatty acid binding proteins can specify the location of the ill intestinal segment, they have been shown to be good indications of intestinal epithelial deterioration [24].

In the current study, ROC curve analysis demonstrated that the cutoff value for the diagnosis of preterm neonates with necrotizing enterocolitis was 7.75 ng/ml with 100% sensitivity and 100% specificity at an AUC of 1.00. The cutoff threshold for intestinal fatty acid binding protein serum levels at diagnosis in detecting the Bell stage in necrotizing enterocolitis was 72.5 ng/ml with a sensitivity of 86% and a specificity of 100% at an AUC of 1.00, according to ROC curve analysis. The AUC for serum I-FABP is 0.92 ng/ml and the AUC for urine I-FABP is 0.81 ng/ml, respectively, according to **Shaaban *et al.* [25],** indicating that they are excellent diagnostic markers with high sensitivity and specificity in the diagnosis of NEC. In light of this, it was discovered that I-FABP levels could separate NEC patients from those who had nonspecific symptoms, correlate with the severity of the disease, and differentiate between surgical and nonsurgical cases [25]. While, study by **Aydemir *et al.* [19]** discovered a cutoff value of 116 pg/ml with a sensitivity of 59% and a specificity of 95%. This threshold might serve as a reference for determining the diagnosis of severe NEC. Additionally, **Abdel-Haie *et al.* [13]** showed in their study that the AUC value for the ROC curve analysis was 0.99. With a sensitivity of 94.4% and a specificity of 100%, the estimated cut-off value for I-FABP for the prediction of NEC was $\geq$7.75 ng/ml at birth. While, the I-FABP cut-off point for predicting NEC is thought to be $\geq$37.95 ng/ml at diagnosis with 100% sensitivity and 100% specificity. I-FABP is estimated to be $\geq$131.8 ng/ml in the detection of NEC stage at diagnosis of NEC, with 90% sensitivity and 100% specificity, according to the ROC curve for serum.

LIMITATION OF THE STUDY

The patient population's modest size. NEC is seen as a separate identity in neonates, whether they are preterm or full-term as the gestational age of every infant in our sample was under 37 weeks.

CONCLUSIONS

Serum level of I-FABP was substantially higher in preterm neonates with NEC than in healthy preterm infants, and it was strongly positively linked with the severity of the condition. It is a sensitive and highly specific protein for NEC harm. Plotting the ROC curve showed that it is a highly effective test for the diagnosis of NEC, and that serum level of I-FABP can serve as a reliable serologic biomarker for the early detection and diagnosis of NEC as well as the assessment of the disease's severity in preterm neonates.

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