RESEARCH PAPER

Urinary Intestinal Fatty Acid Binding Protein for Diagnosis of Necrotizing Enterocolitis

AMARNATH SARAN[1], DEVANANDA DEVEGOWDA[2] AND SRINIVASA MURTHY DORESWAMY[1]
From Departments of [1]Pediatrics and [2]Biochemistry, JSS Medical College, JSS Academy of Higher Education and Research, Mysuru, Karnataka, India.

Corresponding to: Dr Srinivasa Murthy Doreswamy, Professor in Pediatrics, JSS Medical College, JSS Academy of Higher Education and Research, Mysuru 570001, Karnataka, India. drdsrinivasa@gmail.com
Submitted: February 10, 2019; Initial review: June 10, 2019; Accepted: April 05, 2020.

Objective: This study was conducted to compare the urinary levels of intestinal fatty acid binding protein (I-FABP) and I-FABP: Cr (creatinine) between neonates with necrotising enterocolitis and gestation matched healthy controls. **Methods:** 24 neonates with stage 1, 25 with stage 2 and 3 necrotizing enterocolitis, and 25 gestation matched (32.9 wk) controls were compared. Single spot urine sample was collected for estimating the IFABP and creatinine levels. **Results**: Median (IQR) value of urinary I-FABP were higher in those with stage 2, 3 NEC [2773 (2417.7- 2820)] than stage 1 NEC [1164 pg/mL (1341.5 – 2213.4)] and controls [413 (113 – 729.7); pg/mL] (P<0.001). Urinary I-FABP: Cr levels of 3.6 pg/mmoL had a sensitivity and specificity of 96% and 99.5%, respectively in diagnosing stage 2/3 NEC. **Conclusion**: Urinary IFABP: Creatine ratio of 3.6 pg/mmoL is highly specific for stage 2 and 3 NEC.

Key words: *Diagnostic marker, Necrotizing enterocolitis. Newborn, Bell criteria, Preterm*

Published online: **June 12, 2020;** ***PII:*** **S097475591600193**

Necrotizing enterocolitis (NEC) has a reported incidence of 5-10% in very low birth weight babies [1]. Clinical signs and abdominal *X*-ray in early stages are nonspecific, resulting in a diagnostic challenge.

Intestinal fatty acid binding protein (I-FABP) is a sensitive and specific biochemical marker for NEC [2-5]. I-FABP is an intestinal mucosal protein, which escapes into circulation upon enterocyte injury in trauma, infection or ischemia [7-9]. It gets filtered into urine which can be measured to compare the urinary levels of I-FABP and I-FABP clearance between neonates with suspected NEC and gestation matched controls.

METHODS

This study was conducted in a tertiary care unit in India between February, 2015 and December, 2016. Both preterm and term babies with NEC as per modified Bell's criteria were included as cases. Asymptomatic babies with corrected gestational age within 3 days of the cases formed the control group. Babies with surgical problems and cyanotic heart disease were excluded.

Cases were recruited separately in two groups, suspected (stage 1) NEC and established (stage 2 and 3) NEC, for a single control. This study was approved by institutional ethics board. Informed written consent was taken from the parents prior to obtaining urine sample.

Five milliliters of urine sample was collected within 12 hours of diagnosis and stored after centrifugation at –80°C under sterile condition. Sample from an appropriate control was collected and similarly stored. Human I-FABP was quantified using commercially available enzyme linked immunosorbent assay (ELISA) (Hycult Biotech, the Netherlands). Urinary creatinine (Cr) was measured at the same time for estimation of I-FABP: Cr. The incidence of NEC in our unit was 3.7%. Considering specificity of 94% and alpha error of 5%, a total of 75 subjects were needed.

Statistical analysis: Statistical analysis was done using Microsoft Excel 2016 and Analyse-it v4.65.2. Kruskal Wallis test was used to compare the medians and chi-square for proportions. ROC curve was plotted to calculate the sensitivity and specificity of the test.

RESULTS

A total of 74 babies (25 control group, 24 with suspected NEC group and 25 with established NEC) were recruited. Two babies were of 38 weeks gestation and rest were preterms. The baseline characteristics are depicted in ***Table* I**. Ten babies (6 with established NEC) received CPAP/ventilator support and none had birth asphyxia.

Median (IQR) urinary I-FABP value and urinary IFABP: Cr in various groups is depicted in ***Table* II**. Urinary IFABP value of 900 pg/mL had a sensitivity of

WHAT THIS STUDY ADDS

- Raised urinary intestinal fatty acid binding protein (I-FABP) and I-FABP: Creatinine ratio are sensitive and specific for the diagnosis of necrotizing enterocolitis in neonates.

Table I Baseline Characteristics of the Study Population

	Control (n=25)	*Suspected NEC (n=24)*	*NEC stage 2 or 3(n=25)*
#Gestation (wk)	32.9 (2.3)	32.8 (2.3)	32.9 (2.3)
*Birthweight (kg)	1.7 (1.45-1.9)	1.56 (1.36-1.89)	1.54 (1.17-1.81)
Male, *n* (%)	14 (56)	13 (54.2)	18 (72)
Breast milk	23 (92)	24 (100)	24 (96)
Normal delivery	9 (36)	6 (25)	9 (36)

*P>0.05 for all inter-group comparisons; All values in no. (%) except #mean (SD) *median (IQR).*

91.8% and specificity of 92% in diagnosing stage 1 NEC and a value of 1800pg/mL had a sensitivity of 88% and specificity of 82% in diagnosing stage 2 and stage 3 NEC.

Urinary I-FABP: Cr ratio of 2.1pg/mL had a sensitivity of 83.3% (95% CI 66.4, 95.3%) and specificity of 96% (95% CI 87, 99.8%) for the diagnosis of stage 1 NEC. True positive rate, true negative rate, positive likelihood ratio and negative likelihood ratio were 91.8% (79.5-97.3%), 96% (77%-99.7%), 11.3 (4.4-28.9), 0.04(0.006-0.28), respectively. A higher ratio of 3.6 pg/mmoL had a sensitivity of 96% (95% CI 69.7, 97%) and specificity of 99.5% (95% CI 87.2, 99.8%) for diagnosis of stage 2 and 3 NEC; area under the curve was 0.99%. True positive rate, true negative rate, positive likelihood ratio and negative likelihood ratio were 96% (77.6-99.7%), 93.8% (82-98.4%), 24 (3.5-164), 0.06 (0.02-0.19), respectively.

DISCUSSION

A reliable marker for intestinal injury can be helpful in clinically challenging situations. Our study showed a significant increase in urinary excretion of I-FABP in babies with NEC.

Several earlier studies have shown that both plasma and urinary I-FABP are useful markers in neonatal NEC [8-13]. Serial urinary and plasma IFABP values predicted the progression and complication within 8 to 16 hours after onset of symptoms, and the highest level was seen by 24 hours [8]. Neonates with higher baseline values had developed NEC unlike others with lower values [11].

Urine I-FABP levels were also found specifically elevated in NEC and not in sepsis [14]. A urinary IFABP value of 1000 pg/mL was found to have a sensitivity of 100% and specificity of 83% for diagnosis of bowel ischemia [15], similar to our study.

Earlier studies have reported cut-off values of IFABP: Cr ratio ranging from 2 to 5 pg/mmoL for diagnosis of NEC [19,14]. The variations in cut-off values is due to different priorities regarding sensitivity and specificity, and the sample size. Our study showed high specificity, and has good sensitivity for urine IFABP: Cr level of 3.6 pg/mmoL for stage 2 and 3 NEC. As diagnostic tests are done on clinical suspicion, even if the pre test probability is about 50%, the post test probability would be greater than 98%.

The present study did not prospectively collect data with regard to respiratory support, sepsis or asphyxia, which may be considered as a study limitation.

To conclude, urinary I-FABP is significantly elevated in neonates with NEC. Urinary I-FABP: Creatine ratio performed better than urinary I-FABP alone for diagnosis of NEC and can serve as a quantitative marker for assessment of severity of the illness.

Contributors: Contributors: AS: recruited the subjects, collected the data, carried out literature review and prepared the initial draft of manuscript; DD: conducted the biochemical test and contributed to manuscript writing; SMD: conceived and designed the study, analyzed the data, and finalized the manuscript. All authors have approved the manuscript submitted.

Table II Urinary I-FABP levels and I-FABP: Creatinine in Neonates with Necrotizing Enterocolitis (*N*=74)

	Control (n=25)	*Suspected NEC stage 1(n=24)*	*NEC 2 or 3 (n=25)*
Urinary IFABP, pg/mL	413 (113-729.7)	1164 (1341.5-2213.4)	2773 (2471.7-2820)
Urinary IFABP: Creatine, pg/mmoL	1.23 (0.56-1.57)	2.78 (2.32-3.36)	4.84 (4.67-5.34)

Data in median (IQR); I-FABP: intestinal fatty acid bindings protein; data in median (IQR); P<0.001 for inter group comparisons.

Funding: None; *Competing interests*: None stated.

REFERENCES

1. Muller MJ, Paul T, Seeliger S. Necrotizing enterocolitis in premature infants and newborns. J Neonatal Perinatal Med. 2016;9:233-42.
2. Reisinger KW, Van der Zee DC, Brouwers HA, Kramer BW, van Heurn LW, Buurman WA, *et.al*. Noninvasive measurement of fecal calprotectin and serum amyloid – A combined with intestinal fatty acid-binding protein in necrotizing enterocolitis. J Pediatr Surg. 2012;47:1640-5.
3. Funaoka H, Kanda T, Fujii H. Intestinal fatty acid-binding protein (I-FABP) as a new biomarker for intestinal diseases. Rinsho Byori. 2010;58:162-8.
4. Yang G, Wang Y, Jiang X. Diagnostic value of intestinal fatty-acid-binding protein in necrotizing enterocolitis: A systematic review and meta-analysis. Indian J Pediatr. 2016;83:1410-9.
5. Aydemir C, Dilli D, Oguz SS, Ulu HO, Uras N, Erdeve O, *et.al*. Serum intestinal fatty acid binding protein level for early diagnosis and prediction of severity of necrotizing enterocolitis. Early Hum Dev. 2011;87:659-61.
6. Smathers RL, Petersen RD. The human fatty acid-binding protein family: Evolutionary divergences and functions. Human Genomics. 2011;5;170-91.
7. Bingold TM, Franck K, Holzer K, Zacharowski K, Bechstein WO, Wissing H, *et.al*. Intestinal fatty acid binding protein: A sensitive marker in abdominal surgery and abdominal infection. Surg Infect (Larchmt). 2015;16:247-53.
8. Schurink M, Kooi EM, Hulzebos CV, Kox RG, Groen H, Heineman E, *et.al*. Intestinal fatty acid-binding protein as a diagnostic marker for complicated and uncomplicated necrotizing enterocolitis: A prospective cohort study. PLoS One. 2015;10:e0121336.
9. Evennett NJ, Hall NJ, Pierro A, Eaton S. Urinary intestinal fatty acid-binding protein concentration predicts extent of disease in necrotizing enterocolitis. J Pediatr Surg. 2010;45:735-40.
10. Gregory KE, Winston AB, Yamamoto HS, Dawood HY, Fashemi T, Fichorova RN, *et.al*. Elevated urinary I-FABP predicts necrotizing enterocolitis within seven days prior to clinical onset. J Pediatr. 2014;164:1486-8.
11. Derikx JP, Evennett NJ, Degraeuwe PL, Mulder TL, van Bijnen AA, van Heurn LW, *et.al*. Urine based detection of intestinal mucosal cell damage in neonates with suspected necrotising enterocolitis. Gut. 2007;56:1473-5.
12. Thuijls G, van Wijck K, Grootjans J, Derikx JP, van Bijnen AA, Heineman E, *et al*. Early diagnosis of intestinal ischemia using urinary and plasma fatty acid binding proteins. Ann Surg. 2011; 253:303-8.
13. Thuijls G, Derikx JP, van Wijck K, Zimmermann LJ, Degraeuwe PL, Mulder TL, *et.al*. Non-invasive markers for early diagnosis and determination of the severity of necrotizing enterocolitis. Ann Surg.2010;251: 1174-80.
14. Coufal S, Kokesova A, Tlaskalova-Hogenova H, Snajdauf J, Rygl M, Kverka M. Urinary intestinal fatty acid-binding protein can distinguish necrotizing enterocolitis from sepsis in early stage of the disease. J Immunol Res. 2016;2016:5727312.
15. Cronk DR, Houseworth TP, Cuadrado DG, Herbert GS, McNutt PM, Azarow KS. Intestinal fatty acid binding protein (I-FABP) for the detection of strangulated mechanical small bowel obstruction. Curr Surg.2006; 63:322-5.