Research Article

Urinary Intestinal Fatty Acid Binding Protein "IFABP" in maturation of the gut in preterm babies.

Samir T. Abd-Allah*, Hanan M. Kamel, Madeha A. Sayed*and Yossra S. Fadle***
* Department of Pediatrics, Faculty of Medicine, Minia University, El Minia, Egypt.
** Department of Clinical pathology, Faculty of Medicine, Minia University, El Minia, ,Egypt.

Abstract
Objective: Formula-fed premature babies have a higher incidence of developing necrotizing enterocolitis (NEC) than breast-fed babies which may be caused by breast milk induced gut maturation. The effect of breast milk on maturation of the gut has been widely studied in animal models and recently in humans. **The aim of this study:** is to evaluate the effects of breast-feeding on maturation of the intestine in premature babies by measuring the postnatal values of a specific enterocyte marker which is urinary intestinal fatty acid binding protein (I-FABP). **Methods**: Maturation of the gut was studied in 60 premature babies (<37 weeks of gestation) without gastrointestinal morbidity. 30 of them were exclusively breast-fed and the other 30 were formula-fed. Urinary I-FABP levels as the measure of gut maturation were measured at 7th, 12th, and 22nd post-natal days. **Results**: In breast-fed babies, there was a statistically significant increase in urinary I-FABP levels between 7th and 12th days after birth compared with formula-fed babies ($p< 0.01$). **Conclusions**: The pattern of postnatal changes in urinary I-FABP levels suggests a delayed physiological response causing significantly delayed gut maturation in formula-fed babies compared with breast-fed ones.
Key Words: breast-feeding, formula feeding, intestinal fatty acid binding protein, gut maturation, mucosal damage, necrotizing enterocolitis.

Introduction
Human milk (HM; milk from the infant's own mother) feedings during the Neonatal Intensive Care Unit (NICU) hospitalization reduce the risk of prematurity-related morbidities in a dose-response manner for very low birth weight babies.(1)

These morbidities include late onset sepsis, necrotizing enterocolitis, chronic lung disease, retinopathy of prematurity, prolonged NICU hospitalization ,increased health care costs , and long-term health and educational problems.(2)

Breast milk is a known source of molecules that act synergistically to protect the gut barrier and enhance the maturation of the gut-related immune response. So During the perinatal period, nutrition is the principal contributor for immunological and metabolic development, and microbiological programming.(3)

Breast milk is the gold standard for preterm nutrition and influences the development of intestinal microbiota and immune system through its bioactive components.(4)

Preterm infants altered gut microbiota interaction(2) with an immature immunologic intestinal response triggers proinflammatory and counter-inflammatory cytokine response. Necrotizing enterocolitis (NEC) is the most common gastro- intestinal emergency in the neonatal intensive care unit (NICU) which is due to excessive inflammatory response against commensal bacteria by the immature intestine following mucosal injury in the postnatal period. (5)

Its prevalence is largely related to birth weight and gestational age (G.A.) with approximately 1 in 10 very low birth weight infants (<1500 g) developing NEC.(5)
Breast-fed newborns are protected against NEC development through improved gut maturation and because there is an estimated 3 to 10 folds risk reduction in infants fed with breast milk compared with those fed with formula milk (6)

Patients and methods
Sixty preterm babies were enrolled in this study, thirty of them were breast-fed preterm

babies (Group I) while the other thirty newborns were formula-fed ones (Group II).
All of the babies admitted to the NICU of the Minya University Hospital of children between August 2015 and March 2016 were eligible for participation.

Patients were included if they met the following inclusion criteria: <37 weeks of gestation, first enteral feeding within 7 days after birth, and diet consisting of either exclusively breast milk or exclusively formula milk. The only exclusion criterion was development of significant gastrointestinal pathology during the 30-day study period, defined as disease of the gastrointestinal tract necessitating surgery, antibiotic treatment, cardiopulmonary support, or discontinuation or reduction of enteral feeding.

Initiation of feeding and advancement of feeding volumes were realized according to the local protocol. The standard guidelines consisted of early initiation of oral feeding within few days after birth depending on the infant's gestational age and general condition.

Feeding volume was increased gradually and discontinued if there were signs of feeding intolerance including bilious gastric retentions, abdominal distention, emesis, or bloody stools.
Sample collection :1 -blood samples: 5 ml of venous blood samples were taken for complete blood count, Total and direct bilirubin , and CRP using fully automated chemical auto-analyzer Dimension-ES, USA.
2- Urine samples: Urine samples were collected on the 7^{th}, 12^{th} and 22^{nd} day after birth.

Samples were collected either from a urine bag connected to an indwelling catheter or from a cotton wool swab placed in the diaper and squeezed through a syringe barrel into a collection tube. Samples were then frozen at -20∘C till the time of analysis.
Urinary I-FABP levels were measured by ELISA.

Statistical analysis

The numerical data were presented as means – standard deviations while non numerical data were presented as percentage. Two tailed-tests were used to analyze differences between the two groups.

P-values less than 0.05 were considered statistically significant. The magnitude of correlations was determined by Pearson's correlation coefficient.

All the data were analyzed by statistical package Prism 3.0 (GraphPad software, SanDiego, CA, USA).Figures were done by Microsoft Office Excel 2007

Results

In the present study, there was a significant statistical difference between breast-fed and formula-fed preterm babies regarding Duration of NICU admission in days ($p <$ 0.01**)which was higher in formula-fed preterm babies compared to breast-fed ones, while there was no significant statistical difference between the two groups of patients regarding gestational age (mean±SD 33.1 ± 2.2, 32.9 ± 2.1 respectively), sex ($p< 0.01$), and birth weight (mean±SD 0.9 ± 0.7,0.1 ± 0.6, -0.97±0.4 respectively).($p< 0.01$ *(Table1)*

There was significant higher incidence of Co-morbidities like respiratory distress and sepsis in formula-fed preterm babies compared to breast-fed ones ($p< 0.01$) for both. Seventy three percent of obese children 26.3% of over weight ones were having NAFLD. *(Table 2)*

Regarding Signs of feeding intolerance between groups in the form of stopping of feeding, their frequency, and duration, all were significantly higher in in formula-fed preterm babies compared to breast-fed ones ($p< 0.01$) for all. Serum platelets were compared to healthy ones (mean±SD for ALT 71.3 ± 21.4, 41.3 ± 19.1, 30.3 ± 4.4 and Serum TLC (mean±SD for AST 69.8 ± 24.5,36.8 ± 5.5, 30.0 ± 4.4 respectively) ($p< 0.01$ and 0.05 respectively). staff CRP mean±SD for AST 69.8 ± 24.5,36.8 ± 5.5, 30.0 ± 4.4 respectively) ($p< 0.01$ *(Table 3)*

There was negative correlation between BMI,weight, cholesterol, TG and ALT and serum visfatin levels. ($p<0.01$ for all). *(Table 4)*

Table (1): Demographic data between groups.

Variable		Group (I) Breast fed babies (n=30)	Group (II) Formula fed babies (n=30)	P. value (Sig.)
Gestational age (wks.)		33.1 ± 2.2(29-36)	32.9 ± 2.1(29-36)	0.71^{NS}
Sex	**Male**	12 (40.0%)	14 (46.7%)	0.60^{NS}
	Female	18 (60.0%)	16 (53.3%)	
Birth weight (gm)		1973 ± 273 (1400-2400)	1955 ± 337 (1450-2700)	0.88^{NS}
Duration of NICU admission (days)		22.1 ± 4.3	26.0 ± 4.8	**< 0.01****

Table (2): Signs of feeding intolerance between groups.

Variable		Group (I) Breast fed babies (n=30)	Group (II) Formula fed babies (n=30)	P. value (Sig.)
Stop feeding	**No**	21 (70.0%)	8 (26.7%)	**< 0.01****
	Yes	9 (30.0%)	22 (73.3%)	
No. of episodes		0.63 ± 1.06 (0-3)	1.83 ± 1.53 (0-5)	**< 0.01****
Duration of episodes (days)		0.83 ± 1.48 (0-5)	1.90 ± 1.54 (0-5)	**< 0.01****
Abd. X-ray findings for intolerance	**Negative**	30 (100.%)	22 (73.3%)	**< 0.01****
	Positive	0	8 (26.7%)	

Table (3): Laboratory data between groups

Variable		Group (I) Breast fed (n=30) (M ± SD)	Group (II) Formula fed (n=30) (M ± SD)	P. value (Sig.)
Hb (g/dl)		17.4 ± 2.21	17.3 ± 2.20	0.83^{NS}
TLC (10^9/L)		10.52 ± 4.73	13.91 ± 4.25	**<0.01****
PLT (10^9/L)		129.4 ± 37.7	97.7 ± 32.4	**<0.01****
Total bilirubin (mg/dl)		6.80 ±2.32	6.83 ±2.43	0.96^{NS}
Direct bilirubin (mg/dl)		0.60 ±0.25	0.58 ±0.24	0.76^{NS}
Staff (%)		1.78 ±1.68	8.47 ± 4.83	**<0.01****
CRP	**Negative**	21 (70.0%)	7 (23.3%)	**<0.01****
	Positive	9 (30.0%)	23 (76.7%)	

Table (4): Comparison between groups regarding IFABP level at different postnatal days.

Variable	Group (I) Breast fed (n=30) (M ± SD)	Group (II) Formula fed (n=30) (M ± SD)	P. value (Sig.)
IFABP (7th day), (ng/l)	3807 ± 319	3040 ± 722	**<0.01****
IFABP (12th day), (ng/l)	3999 ± 735	3262 ± 552	**<0.01****
IFABP (22th day), (ng/l)	3731 ± 828	3414 ± 942	0.17NS
P. value (Sig.)	0.28NS	0.16NS	-

Discussion

FABPs is a set of widely expressed cytoplasmic proteins with small molecular weight and excellent organ specificity, which are immediately secreted into the systemic circulation upon the damage of cells[7]

As a member of the FABPs family, FABP2, which is a FABP2 gene encoding protein, accounts for up to 2% of the cytoplasmic proteins in the mature enterocyte, and it is responsible for the intake alongside with the transport of polar lipids like fatty acids from the lumen of the small bowel.[8]

FABP2 is a water soluble cytosolic protein with a small molecular weight of 14-15 kDa, and it is initially located in the mature enterocytes of the small intestine. FABP2 is also named as intestinal-type FABP (I-FABP).[9]

Because of its small molecular size, FABP2 is believed to be delivered to the circulation immediately upon the loss of the integrity of the cell membrane and filtering of the glomerulus with a renal excretion of 28% and a considerable half-life of 11minutes. So, it is supposed to be detectable in urine.[10]

Thus, varying FABP2 expressions in the urine could exactly reflect the severity of the cell damage to the intestinal epithelia, making it possible to use FABP2 as a trustable indicator of the disease progression[11].

This study was carried out to assess the diagnostic utility of the urinary I-FABP levels as a new marker for gut maturation in breast-fed preterm neonates compared to formula-fed ones.

In the present study, breast-fed babies and formula-fed ones showed no significant statistical difference regarding gestational age (p=0.71), sex (p< 0.60) and birth weight (p< 0.88). These findings agreed with the study of Kostan W 2014 who stated that there were no significant differences in GA, birth weight, or sex between the 2 groups; however, there was a trend of lower median GA in the breast-fed group.

Our study shows that Prematurity was the primary reason for admission to the NICU in all of the babies and that the duration of NICU admission in days was higher in formula-fed preterm babies compared to breast-fed ones (p **< 0.01)**.

The results of our study show a significant higher incidence of Co-morbidities like respiratory distress and sepsis in formula-fed preterm babies compared to breast-fed ones (p< 0.01) for both . This agrees with the study of [12] who explained the beneficial effects of breast milk and its immunomodulatory and anti-inflammatory effect, and high concentrations of secretory immunoglobulin A, CD14, transforming growth factor-b, erythropoietin, and interleukin-10 in breast milk.

In our study, total volume of enteral feedings was recorded every day to investigate whether the type of feeding correlated with feeding

intolerance**.** Feeding intolerance is defined as episodes of discontinuation of enteral feeding or frequency and cumulative amount of gastric retentions.

We found that signs of feeding intolerance including feeding stoppage, number of episodes, and duration of those episodes were significantly higher in in formula-fed preterm babies compared to breast-fed ones ($p< 0.01$) for all**.** . This could be explained by that early breast milk feedings, especially colostrum, promote the growth, maturation, and protection of the gut epithelial border.

In agreement with our study,[13] explained the mechanisms by which breast-feeding improves intestinal maturation. For example, analogues of growth factors or human milk oligosaccharides. The preterm infant's high need for trophic factors should be taken into account. Added to that, his study underlines the importance of breast milk use in preterm infants.

Our results also agree with the study of [14] who found that human milk feedings have been shown to stimulate healthy gut microflora, reduce intestinal permeability, and interfere with the translocation of bacteria from the gut lumen to the mucosa, and appear to be the most critical as VLBW infants transition from intrauterine (e.g., swallowing amniotic fluid) to extrauterine nutrition in the early post-natal period.

Commercial formulas may have a separate detrimental impact on these processes during these early postnatal exposure periods, via up-regulation of inflammatory processes, GIT epithelial cell toxicity, and other mechanisms.[15]

References

1. Meier, P. P., Engstrom, J. L., Patel, A. L., Jegier, B. J., & Bruns, N. E.. Improving the use of human milk during and after the NICU stay. Clinics in perinatology, (2010); 37(1), 217-245.
2. Fullerton, B. S., Hong, C. R., Velazco, C. S., Mercier, C. E., Morrow, K. A., Edwards, E. M.,... & Jaksic, T.. Severe neurodevelopmental disability and healthcare needs among survivors of medical and surgical necrotizing enterocolitis: a prospective cohort study. Journal of pediatric surgery, (2018); 53(1), 101-107.
3. Mills, S., Stanton, C., Lane, J. A., Smith, G. J., & Ross, R. P.. Precision nutrition and the microbiome, Part I: Current state of the science. Nutrients, (2019) 11(4), 923.
4. Collado, M. C., Cernada, M., Neu, J., Pérez-Martínez, G., Gormaz, M., & Vento, M.. Factors influencing gastrointestinal tract and microbiota immune interaction in preterm infants. Pediatric research, (2015 77(6), 726.
5. Maheshwari, A., Corbin, L. L., & Schelonka, R. L.. Neonatal necrotizing enterocolitis. Research and Reports in Neonatology, (2011)1, 39.
6. Cilieborg, M. S., Boye, M., & Sangild, P. T.. Bacterial colonization and gut development in preterm neonates. Early human development, (2012)88, S41-S49.
7. Briana DD, Liosi S, Gourgiotis D, Boutsikou M, Baka S, Marmarinos A, et al., Cord blood intestinal fatty acid-binding protein (I-FABP) in full-term intrauterine growth restricted pregnancies. J Matern Fetal Neonatal Med 2012; 25:2062-2065
8. 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;87:659-661
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;45:735-740.
10. Reisinger KW, Derikx JP, Thuijls G, van der Zee DC, Brouwers HA, van Bijnen AA, et al., Noninvasive measurement of intestinal epithelial damage at time of refeeding can predict clinical outcome after necrotizing enterocolitis. Pediatr Res 2013; 73:209-213
11. 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; 251:1174-1180.
12. Walker A. Breast milk as the gold standard for protective nutrients. J Pediatr 2010; 156:S3–7.
13. Quigley MA, Henderson G, Anthony MY, et al., Formula milk versus donor breast

milk for feeding preterm or low birth weight infants. Cochrane Database Syst Rev **2007CD002971**

14. Taylor SN, Basile LA, Ebeling M, Wagner CL. Intestinal permeability in preterm infants by feeding type: Mother's milk versus formula. Breastfeed Med. 2009 Mar; 4(1):11–5. [PubMed: 19196035]
15. Newburg DS. Innate immunity and human milk. Journal of Human Lactation. 2005