Oral Probiotics Reduce Incidence of Necrotizing Enterocolitis and Sepsis in Neonates with Hypoxic Ischemic Encephalopathy

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Abstract. ***Objective.*** We evaluated the efficacy of probiotics in reducing the incidence of feeding intolerance, necrotizing enterocolitis (NEC) and sepsis in full-term, bottle fed hypoxic ischemic encephalopathy (HIE)infants and verifies the viability of probiotic bacteria *Lactobacilli* in the stool and fecal IgA when lyophilized *Lactobacillus acidophilus*(*L. acidophilus*) was fed to those infants. ***Patients and Methods.*** A prospective, double blind, randomized, controlled clinical trial was conducted on 60 full-term newborns suffering from HIE grade II or III. They were randomized into 2 groups; the infants in the probiotic group were fed with lyophilized *L. acidophilus* in dose of 6 x 10^9 CFU divided into 2 doses/day in addition to the traditional HIE therapy until discharged while infants in the control group were given the traditional HIE therapy alone. *Lactobacilli* count and fecal IgA were determined at both day 7 and the end of the study. Primary outcome include NEC and sepsis. ***Results.*** A total of 60 HIE neonates were enrolled: 30 in the probiotic group and 30 in the control group. The incidence of NEC and sepsis was significantly lower in the probiotics group (16.7% vs. 33.3%, P=0.034 and 26.7% vs 73.3%, P= 0.0008, respectively). The fecal IgA concentration was significantly increased from day 7 to the end of the study in the probiotic group (6.9±1), but not in the control group (2.8±1.2, P<0.0001).***Conclusion.*** The supplementation of lyophilized *Lactobacilli acidophilus* in full-term newborns with HIE grades (II,III) was beneficial, as it enhances the tolerability to enteral feeding and decreases the incidence of feeding intolerance, NEC and sepsis in our neonates.
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1.Introduction

Hypoxia in the perinatal period is an important cause of multiple disabilities in neonates [1]. If hypoxia affected the brain, it leads to hypoxic ischemic encephalopathy (HIE) which may lead to cerebral palsy and epilepsy[1], HIE is the second cause of infant mortality in Egypt after sepsis[2].

HIE neonates have a greater incidence of feeding difficulties, necrotizing enterocolitis (NEC) and neonatal sepsis (NS) [3].NEC is among the most common life-threatening gastrointestinal emergency experienced in neonates and one of the most difficult to eradicate[3]. It is associated with significant morbidity and mortality[4]. Neonatal sepsis is a clinical syndrome characterized by systemic signs of infection and accompanied by bacteremia in the first 28 days of life [4].

probiotic bacteria reduce the overgrowth of pathogens in the neonatal bowel, and reduce the infections[5]. Probiotics might reduce the risk of NEC in neonates[5]. Nevertheless, the use of live bacteria raised some concerns about translocation of infection [5]. probiotics are microorganisms administered in adequate amounts that have a beneficial effect on the host, an example is lacteal-forte probiotic which is a unique in that it is the only heat killed, freeze-dried *L. acidophilus*[9]**.** Unlike other probiotics, there is no risk of translocation of infection with lacteal-forte. [9]

2. Patients and Methods:

Patients

This prospective randomized double blind placebo controlled study was conducted at NICU of Tanta University Hospital from March 2011 to July 2012 on 60 full-term newborn suffering from HIE grade II or III. HIE was assessed during 1st 24 hours of life and classified according to the American Academy of Pediatrics guidelines of HIE.[6] NEC is categorized by modified Bell's classification[7]. The diagnosis and classification of NEC was made by 2 independent neonatologists. Sepsis was diagnosed with clinical signs of sepsis and positive blood culture. The study was approved by The Ethics Committee of Faculty of Medicine, Tanta University.

Inclusion criteria: full-term newborn, Apgar scores of ≤ 3 at 5 minutes, delayed first breath, moderate or severe encephalopathy such as lethargy, seizures or hypotonia. Patients were excluded if they were preterm, exposed to postnatal antibiotics, congenital anomalies, mild cases of HIE (grade I) and neonatal sepsis.

The following items were done for each patient: history, clinical examination and laboratory

investigations including Complete Blood Picture, C - Reactive Protein, blood gases

The enrolled neonates were divided into two groups: 30 newborn each. Probiotic (study) group included HIE newborn who were given probiotic lacteol-fort in addition to the traditional therapy of HIE, and Control group included HIE newborn who were subjected to the traditional HIE therapy alone. Neonates in the probiotic group received lyophilized *Lactobacillus* in dose of 6 x 10^9 CFU divided into 2 doses/day till reaching full feed[12].while the control group received placebo(distilled water) [5].

Methods

Bacteriological culture:

All raw materials were supplied by Oxoid, UK and prepared in Medical Microbiology and Immunology Department, Faculty of Medicine, Tanta University according the manufacturer's instructions.

Stool culture:

Sampling and processing: Stool samples were collected from each newborn of all groups on admission, at day 7 and at the end of the study to identify and count *Lactobacilli*. Additionally, stool samples were collected on suspicion of NEC. Stool samples were collected in clean and dry containers then were transported immediately to the laboratory to be examined as early as possible[12]. For *Lactobacilli* isolation, stool samples were processed according to **Riskin *et al.,***[8]. Briefly, one gram of feces was emulsified in 9 ml sterile physiological saline (representing 1:10 dilution) then was vortex well. The sample was further diluted to 1:100 and 1:1000 using sterile physiological saline. Twenty microliter from each diluted sample was streaked on Man Rogosa (MRS) agar plates, placed in candle jar in an atmosphere enriched with 5-10% CO_2 and incubated at 37˚C for 72 hours. For isolation of pathogenic organisms, stool samples were processed according to **Collee *et al.,***. [9]. Stool culture was done on appropriate culture media and incubated at 37˚C for 24-48 hours and any growth was identified according to the standard microbiological protocol[9].

Identification and enumeration of Lactobacilli:

The developed colonies were picked up and identified in a systemic manner based on cell morphology, analysis of fermentation products and associated enzyme activities[9]. Enumeration of *Lactobacilli* was done by plate count technique on MRS plates. After cultivation of each diluted sample on MRS, *Lactobacillus* colonies were identified and typical colonies were counted in each plate then the number was multiplied by the dilution factor and the result was reported as the number of viable bacteria per gram of stool[9].

Stool IgA:

Fecal IgA were measured with the IgA ELISA kits (Immundiagnostik AG, Bensheim, Germany), using 100 mg of fresh feces. The absorbance was measured at 405 nm with a microtiter plate reader.

Statistical analysis:

It was performed by using SPSS for Windows, version 9. Data were expressed as range and mean ± standard deviation (SD)/ or numbers and percentages. Differences between groups in continuous variables were tested for significance with paired t-test while univariate analysis was done with the *Chi-square*test. For all statistical tests done, *P* value< 0.05 was considered significant. Linear Correlation Coefficient was used to assess different correlations.

3.Results

A total of 60 HIE neonates, 30newborn (16 males, 14females, aged 1 to 4 days; mean ± SD: 2.33+1.17, mean birth weight: 3+0.60) had received traditional therapy of hypoxia while 30 newborn (18 males, 12 females, aged 1 to 4 days; mean ± SD: 2.40+1.31, mean birth weight: 3+0.63) had received lacteal-fort in addition to traditional therapy of hypoxia with no statistical difference between them (*P*>0.05).

Table 1: Clinical and laboratory parameters in probiotic and traditional therapy groups

Parameters	Probiotic Group (n=30)	Traditional therapy Group (n=30)	t-test	*P*-value
Abdominal girth (cm)	1.8±0.55	2.3±0.96	2.48	0.016*
Prefeed gastric residue (%)	15.75±5.5	20±8.5	2.3	0.025*
Time of full enteral feeding (d)	20±4.4	25.6± 3.2	5.64	<0.0001*
Weight gain (kg)	3.2± 0.65	2.9±0.4	2.15	0.036*
Duration of hospital stay (d)	25.1±2.3	30±4.2	5.6	<0.0001*
Lactobacilli colonies in stools (n)^	$2.6\pm1.4\times10^6$	$0.3\pm0.1\times10^6$	8.96	<0.0001*
Lactobacilli colonies in stools (n)#	$5.6\pm7.3\times10^6$	$2.1\pm1.8\times10^6$	8.63	<0.0001*

Values are mean ± SD. Total bacteria presented as mean ± standard deviation (SD) of log10 colony forming units (CFUs g-1 fresh faecal sample).*$P<0.05$ is significant using paired t-test. ^ No at day 7 of admission.# No at the end of the study (till reach full feeding)

Abdominal girth and prefeed gastric residue showed decrease incidence of abdominal distension and prefeed gastric residue in probiotics group. there was a significant reduction in time to reach full enteral feeding in probiotic group as compared with control group (P<0.0001), there was significant increase in weight gain in probiotic group as compared with control group and decrease in duration of hospital stay in the probiotic versus control group (P<0.0001).

Regarding NEC and NS, there was significant difference between the probiotic and traditional therapy (control) groups (p=0.034 and 0.0008) with obvious decreased incidence in the probiotic group (Figs 1a and b).

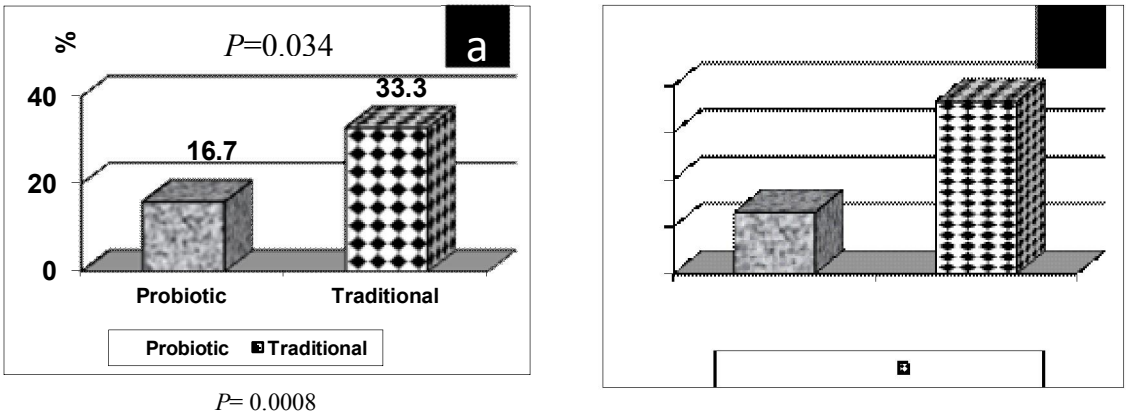

Fig. 1: incidence of necrotizing enterocolitis (NEC) (a) and neonatal sepsis (NS) (b) in probiotic group compared to traditional therapy group

Negative correlation was observed between the lactobacilli count in stool culture and time of full feeding, NEC and sepsis. Similarly, total fecal IgA levels were 50% higher in the probiotic (3.2±0.9) than in the traditional therapy (control group) (2.1± 1.1) at day 7 (t=4.24, P< 0.0001)(Fig. 2a). The fecal IgA concentration was significantly increased from day 7to the end of the study in the probiotic group (6.9± 1), but not in the control group (2.8±1.2, t=14.38, P<0.0001) (Fig. 2b).

Table 2: Correlation between the lactobacilli count in stool culture and time of full feeding, NEC and in probiotics group

Probiotics group	Lactobacilli count at day 7		Lactobacilli count at the end of the study	
	r.	*P* - value	r.	*P* -value
Time of full feeding	-0.369	0.047*	-0.259	0.025*
NEC	-0.241	0.052*	-0.352	0.035*
NS	-0.233	0.055*	-0.247	0.034*

* P< 0.05 is significant. NEC: necrotizing enterocolitis, NS: neonatal sepsis

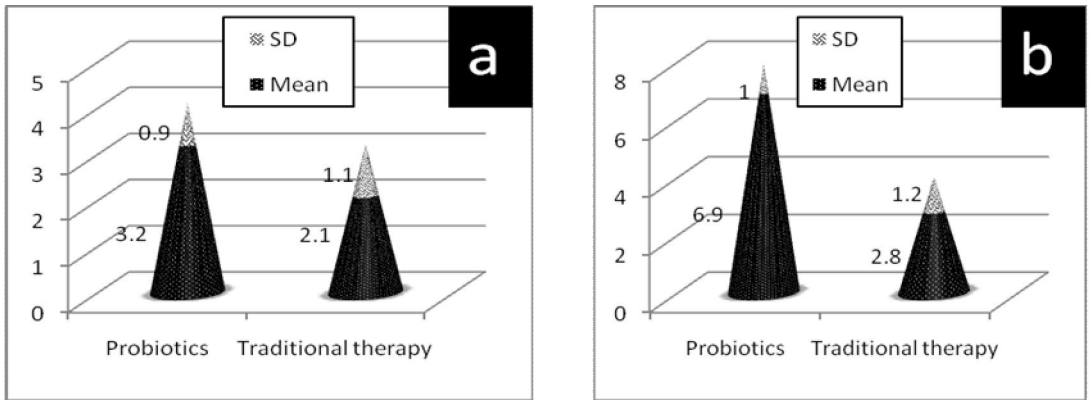

Fig. 2: Fecal IgA level (in milligram per kilogram feces)in the probiotic group compared to the traditional therapy group at day 7 (a) (t=4.24, P < 0.0001) and at the end of the study (b) (t=14.38, P<0.0001).

4.Discussion

To the best of our knowledge, this is the first study to determine the role of lyophilized *L. acidophilus* in preventing feeding intolerance, NEC and sepsis in neonates with HIE.

In our study, babies in the probiotic group were significantly heavier and reached full enteral feeding in shorter time with decrease in duration of hospital stay. **HU XY *et al.,*** observed significant increase in weight and decrease in the time to reach full enteral nutrition in probiotic group versus non-probiotic group[10].

Indrio *et al.*, suggested a useful role for probiotics in improving feeding tolerance in their probiotic group[13].

Awad *et al.*, reached similar conclusion that probiotics were reduced incidence of sepsis and NEC[11]. Similarly,**REN and WANG,** stated that probiotics could decrease diarrhea and sepsis in neonates[14]. **Braga *et al.*,** confirmed that oral supplementation of probiotics reduced the occurrence of NEC[5].

In contrary, **Fernández-Carrocera *et al.*,** had reported that no difference in NEC and sepsis rate between probiotics and non-probiotics groups[15].

Secretory IgA plays a central role in local immunity. the fecal IgA concentration was significantly increased in the probiotic group but not in the control group with positive correlation of fecal IgA with the cell counts of *Lactobacilli* and this in agreement with **Mohan *et al.*,**[16] who observed an increase in the total fecal IgA concentration in the probiotic group whereas there was no change in the concentration in the control group.

Conclusion

Supplementation of probiotics in form of lyophilized L. acidophilus (Lacteol-fort) in dose of (6x109) colony forming units (CFU) in full-term newborns with HIE grade (II,III) was beneficial, as it enhance the tolerability to enteral feeding and decreases the incidence of feeding intolerance, NEC and sepsis. One of the limitations of this study is that identification of a probiotic effect on the growth of probiotic bacteria was based on stool cultures only. Also, none of the other probiotic bacteria, such as bifidobacteria, were grown.

The authors have no conflicts of interest relevant to this article.

References:

1. Huang BY, Castillo M. Hypoxic-ischemic brain injury: imaging findings from birth to adulthood. *Radiographics* 2008; 28 (2): 417-39.
2. El Metwally D. Hypoxic ischemic encephalopathy, still haunting. *Neuropediatrics* 2006; S1 (37) – Pl4_2_2. (Congress abstract)
3. Henry MC, Moss RL. Necrotizing enterocolitis. *Ann Rev Med* 2009; 60:111-24.
4. Oh S, Young C, Gravenstein N, Islam S, Neu J. Monitoring technologies in the neonatal intensive care unit: implications for the detection of necrotizing enterocolitis. *J Perinatol* 2010;30 (11):701-8.
5. Braga TD, da Silva GA, de Lira PI, de Carvalho Lima M. Efficacy of *Bifidobacterium breve* and *Lactobacillus casei* oral supplementation on necrotizing enterocolitis in very-low-birth-weight preterm infants: a double-blind, randomized, controlled trial. *Am J Clin Nutr* 2011;93(1):81-6.
6. American Academy of Pediatrics. Relation between perinatal factors and neurological outcome. In: Guidelines for Perinatal Care. 3rd ed. Elk Grove Village, Ill: American Academy of Pediatrics; 1992: 221-34.
7. Walsh MC, Kliegman RM, Fanaroff AA. Necrotizing enterocolitis: a practitioner's perspective. *Pediatr Rev* 1988;9: 219–26.
8. Riskin A, Hochwald O, Bader D, Srugo I, Naftali G, Kugelman A, Cohen E, Mor F, Kaufman B, Shaoul R. The effects of lactulose supplementation to enteral feedings in premature infants: a pilot study. *J Pediatr* 2010;156(2):209-14.
9. Collee JG, Fraser AG, Marmion BP, Simmons A. Mackie and McCartney practical medical microbiology. New York: Churchill Livingstone 1996.
10. Hu XY, Zhou YX,Xu SZ, Lin YY. Effects of probiotics on feeding intolerance in low birth weight premature infants. *Zhongguo Dang Dai ErKeZaZhi* 2010; 12(9):693-5. (Abstract)
11. Awad H, Mokhtar H, Imam SS, Gad GI, Hafez H, Aboushady N. Comparison between killed and living probiotic usage versus placebo for the prevention of necrotizing enterocolitis and sepsis in neonates.*Pak J Biol Sci* 2010;13(6):253-62.
12. Verdu EF. Probiotics effects on gastrointestinal function: beyond the gut? *Neurogastroenterol Motil* 2009;21 (5):477–80.
13. Indrio F, Riezzo G, Raimondi F, Bisceglia M, Cavallo L, Francavilla R. The effects of probiotics on feeding tolerance, bowel habits, and gastrointestinal motility in preterm newborns. *J Pediatr* 2008;152(6):801-6.
14. REN YF and WANG LL. Effect of probiotics on intestinal bacterial colonization in premature

infants. *Zhongguo Dang Dai ErKeZaZhi* 2010;12 (3) 192-4.

15. Fernández-Carrocera LA, Solis-Herrera A, Cabanillas-Ayón M, Gallardo-Sarmiento RB, García-Pérez CS, Montaño-Rodríguez R, Echániz-Aviles MO. "Double-blind, randomized clinical assay to evaluate the efficacy of probiotics in preterm newborns weighing less than 1500 g in the prevention of necrotizing enterocolitis". *Arch of Dis Child Fetal Ed* 2013; 98 (1): F5-9.
16. Mohan R, Koebnick C, Schildt J, Mueller M, Radke M, Blaut M. Effects of *Bifidobacteriumlactis* Bb12 supplementation on body weight, fecal pH, acetate, lactate, calprotectin, and IgA in preterm infants. *Pediatr Res* 2008; 64: 418–22.

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