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 10⁹ 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. [Mohamed S Elfrargy, Azza M. Hassan and Marwa Abd-El-Wahab. Oral Probiotics Reduce Incidence of Necrotizing Enterocolitis and Sepsis in Neonates with Hypoxic Ischemic Encephalopathy. J Am Sci 2014;10(10):6-10]. (ISSN: 1545-1003). http://www.jofamericanscience.org 2

Keywords: Neonates, Probiotics, Necrotizing enterocolitis, Hypoxic ischemic encephalopathy

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 the first 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 $6 \times 10^9$ 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, 30 newborn (16 males, 14 females, aged 1 to 4 days; mean ± SD: 2.33±1.17, mean birth weight: 3±0.63) 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

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Probiotic Group (n=30)</th>
<th>Traditional therapy Group (n=30)</th>
<th>t-test</th>
<th>$P$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abdominal girth (cm)</td>
<td>1.8±0.55</td>
<td>2.3±0.96</td>
<td>2.48</td>
<td>0.016*</td>
</tr>
<tr>
<td>Prefeed gastric residue (%)</td>
<td>15.75±5.5</td>
<td>20±8.5</td>
<td>2.3</td>
<td>0.025*</td>
</tr>
<tr>
<td>Time of full enteral feeding (d)</td>
<td>20±4.4</td>
<td>25.6±3.2</td>
<td>5.64</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>Weight gain (kg)</td>
<td>3.2±0.65</td>
<td>2.9±0.4</td>
<td>2.15</td>
<td>0.036*</td>
</tr>
<tr>
<td>Duration of hospital stay (d)</td>
<td>25.1±2.3</td>
<td>30±4.2</td>
<td>5.6</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>Lactobacilli colonies in stools (n)</td>
<td>2.6±1.4x10$^5$</td>
<td>0.3±0.1x10$^5$</td>
<td>8.96</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>Lactobacilli colonies in stools (n)$^*$</td>
<td>5.6±7.3x10$^5$</td>
<td>2.1±1.8x10$^5$</td>
<td>8.63</td>
<td>&lt;0.0001*</td>
</tr>
</tbody>
</table>

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).

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 7 to 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**

<table>
<thead>
<tr>
<th>Probiotics group</th>
<th>Lactobacilli count at day 7</th>
<th>Lactobacilli count at the end of the study</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$r$.</td>
<td>$P$-value</td>
</tr>
<tr>
<td>Time of full feeding</td>
<td>-0.369</td>
<td>0.047*</td>
</tr>
<tr>
<td>NEC</td>
<td>-0.241</td>
<td>0.052*</td>
</tr>
<tr>
<td>NS</td>
<td>-0.233</td>
<td>0.055*</td>
</tr>
</tbody>
</table>

* $P<0.05$ 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 \textit{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. \textit{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].

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

\textit{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 \textit{et al.}, confirmed that oral supplementation of probiotics reduced the occurrence of NEC[5].

In contrary, Fernández-Carrocera \textit{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 \textit{Lactobacilli} and this in agreement with \textit{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 \textit{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 enhances the tolerance 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:
2. El Metwally D. Hypoxic ischemic encephalopathy, still haunting. \textit{Neuropediatrics} 2006; S1 (37) – P14.2. (Congress abstract)
14. REN YF and WANG LL. Effect of probiotics on intestinal bacterial colonization in premature...
