International Journal of Children and Adolescents

Original Article
IJCA, Vol. 2, No. 4, Oct, 2016.5-9.

Use of Probiotic for the Treatment of Acute Rotavirus Diarrhea in Children: a Randomized Single-Blind Controlled Trial

Behnam Sobouti: Infectious Disease Research Center, Ali-Asghar Children's Hospital, Iran University of Medical Sciences, Tehran, Iran.
Samileh Noorbakhsh: Infectious Disease Research Center, Rasoul-e-Akram Hospital, Iran University of Medical Sciences, Tehran, Iran.
Haleh Ashraf: Department of Cardiology, Tehran University of Medical Sciences, Tehran, Iran.
Shaghayegh Ashraf -Talesh: (**Corresponding author*) Ali-Asghar Children's Hospital, Iran University of Medical Sciences, Tehran, Iran. ha_as_ta@yahoo.com

Received: 25 Feb 2016 Accepted: 20 May 2016

Abstract

Background and Objective: Despite consistent evidence that probiotics reduce the duration of diarrhea, there is only weak evidence for their efficacy in reducing the duration of hospitalization. Another source of heterogeneity for probiotic trials is the type of probiotic being assessed; also, information about combined products is scarce.

Methods: This is a randomized, single-blind controlled clinical trial performed in children hospitalized with acute rotavirus diarrhea carried out at a university hospital in Tehran, Iran. Children were randomly assigned to receive rehydration therapy or the same plus a multi-strain probiotic preparation. The primary outcome was the duration of hospitalization..

Findings: A total of 60 patients with acute diarrhea secondary to rotavirus infection were included; baseline characteristics were similar in both groups. A statistically significant difference in the duration of hospitalization was observed ($p < 0.001$) in children receiving probiotic (5.07 ± 1.30) in comparison to the control group (8.22 ± 2.14).

Conclusion: Results of this study support the use of multi-strain preparation probiotics in treating rotavirus acute diarrhea.

Keywords: Probiotics; Rotavirus, Diarrhea and Children

Introduction

Despite improvements in the treatment and prevention of acute diarrhea which kills 2200 infants and children per day worldwide, this condition still can be particularly detrimental to children especially in the less developed countries (1). Rotavirus represents the most common etiologic agent associated with diarrhea in the children both in developing and developed countries, especially in those countries which has not launched a rotavirus vaccination program. In Iran, overall pooled estimate of infection with rotavirus among cases of gastroenteritis is 35%, and pooled estimates for hospitalized children and outpatient subgroups are 39% and 31%, respectively (2). Different supportive measures including fluid repletion, antiemetic agents have reduced significantly the incidence of mortality and morbidity caused by diarrhea but does not shorten the duration of diarrhea, does not change the consistency of the stools, and it does not normalize gastrointestinal flora. Probiotics are commonly used in viral diarrhea in order to suppress the growth or epithelial invasion of pathogenic bacteria in the human gut, improve the intestinal barrier function, modulate the immune system of the intestine, and mediate analgesic functions (3). Clinical trials have demonstrated the effectiveness of probiotics in the treatment and prevention of acute diarrhea, decreasing the severity and duration of rotavirus infections in children as well as diarrhea in adults. Several probiotic strains have been tested in hospitalized children with different results (4, 5). Despite consistent evidence that probiotics reduce the duration of diarrhea, there is only weak evidence for their efficacy in reducing the duration of hospitalization (6). Alt-

hough data on hospital stay are not conclusive, the use of probiotics in this setting may have significant impact on the health care burden and diarrhea-associated costs. In Iran, studies on the effectiveness of probiotics are not thorough and also there is some controversy (7-10). The majority of these studies appear to support the efficacy of probiotic therapy in the setting of acute diarrhea (8-10), and only one was focused on rotavirus infection but in that study, the test used to detect rotavirus was not ideal, introducing a bias risk in the studied patients (7). Another source of heterogeneity for probiotic trials is the type of probiotic being assessed. Significant differences in effectiveness have been reported for different species and strains of similar species of bacteria and yeasts. In general, information about combined products is scarce(2, 5).

With this in mind, in this study we aimed to test the use of a multi-strain probiotic preparation, Protexin including the child specific strain bifidobacterium infantis added in the management of acute diarrhea.

Methods

A prospective randomized single-blind controlled trial was designed in order to assess the effectiveness of a mixed probiotic preparation and a control group. The study was conducted in the pediatric ward of Rasoul-e-Akram Hospital, Tehran, Iran. The study protocol was approved by the hospital ethics committee and the investigation board of the Pediatric department of Iran University of Medical Sciences. Consent was obtained from parents or caretakers of all the children who met the inclusion criteria for the study before commencing the study protocol. Patients aged from 3 months to 7 years, with a history of acute watery diarrhea positive for rotavirus of less than 72 h duration, who were brought to the gastroenterology or infectious disease department, were eligible for inclusion in the study. The exclusion criteria were moderate to severe malnutrition evaluated according to the WHO tables of growth for children fewer than 5 years, using z-score weight/height (11), severe chronic disease, severe electrolyte disturbances or clinical or other laboratory signs of coexisting diseases such as sepsis, systemic infections requiring antibiotic therapy, immunodeficiency, cystic fibrosis, food allergy or other chronic gastrointestinal diseases, use of probiotics in the previous three weeks, use of antibiotics or any anti-diarrheal medication in the previous three weeks and during the study, and poor compliance (defined by administration of less than four doses of the study medication) antibiotic treatment during the preceding 7 days, known chronic uncontrolled intestinal disease such as celiac disease, pancreatic insufficiency. The identification of another enteric pathogen or mixed enteric infections and ingestion of antibiotics, or probiotics, in the last 3 weeks before admission, were also exclusion criteria.

Procedures

On admission to the study data about full clinical history, physical examination, nutritional status, dehydration, fever, oral tolerance, and stools characteristics were recorded. Parents or caretakers of the enrolled patients were not informed about the preparation assigned to their children. Nursing staff were responsible for the administration of the solution. They were trained to prepare the solutions assigned in similar containers to mask the nature of the product. Patients were randomly assigned from a computerized admissions list to one of the different treatment groups. The probiotic group received a multi-strain commercial preparation (Protexin Compounder, Probiotics International Ltd, UK) that contains a complex blend of seven bacteria *lactobacillus casei, lactobacillus rhamnosus, streptococcus thermophilus, bifidobacterium breve, lactobacillus acidophilus, lactobacillus bulgaricus* and the child specific strain bifidobacterium infantis (1×10^9 CFU/g of *L*) twice a day for at least 5 days. If the child vomited, the administration of the respective repeated after 15 minutes. Any problems with the consumption of the products were reported immediately to the principal investigator.

The control group was assigned to receive oral or systemic rehydration solutions alone, according to the evolution of diarrhea. It should be noted that the probiotic group also received oral or systemic hydration according to the disease evolution. All the patients were fed according to their age. Those aged less than 6 months were breastfed or received milk formula appropriate for their age. Patients aged more than 6 months received milk formula or breast milk and solid food based on chicken meal, potatoes, rice, carrots, and vegetable oil. No patient included in the study was administered delactosed milk, supplemental vitamins, or zinc.

Within three hours of admission at the most the following laboratory tests were performed on all participants: blood count (including measurement of hemoglobin, peripheral white cell count and differential formula), erythrocyte sedimentation rate (ESR) a semi-quantitative C-reactive protein. Parasitological assessment by PAF technique by

Lugol method (12). Stool specimens were obtained as early as possible after admittance to the hospital and were examined for stool cultures, microscopic examination for bacterial pathogens and parasites and rotavirus antigen. Stool cultures were performed for enteropathogenic, toxigenic, enteroadherent, enteroaggregative, invasive and enterohaemorrhagic E. coli and for Salmonella typhi and Shigella spp using routine procedures.

The test used for the diagnosis of rotavirus and adenovirus was based on the polymerase chain reaction assay (PCR, Roche, Germany). The nursing staffs were in charge of recording the number and consistency of stools. Probable adverse effects were also recorded on previously designed charts. Patients were re-evaluated at the Outpatient Department 24 to 48 hours after discharge. The primary outcome of the study was the duration of the diarrhoea measured from the time of admission until the first normal evacuation was detected.

Definitions

Acute diarrhea was defined as the passage of 3 or more liquid stools and/or a change of consistency from normal to liquid but without visible blood or an increase above the normal daily frequency of evacuations in a 24-hour period (3). Time of hospitalization was considered the interval of time from admission until discharge. Hospital discharge was considered 24 h after resolution of diarrhea was achieved.

The clinical scale of Fortin and Parent was employed to classify the degree of dehydration into mild, moderate, or severe (13).

Statistical Analysis

Data were analyzed using SPSS software (version16.0). Binary and ordinal data were summarized using percentages and compared using x2 test and Fisher's exact test when required. Continuous (Scale) data are presented as mean ± standard deviation (SD) or median (interquartile range) and compared using the Mann–Whitney U test or independent-samples T test, as appropriate. Pearson's and spearman correlation analysis were utilized to determine unadjusted bivariate correlation between two variables. A multivariate linear regression analysis was also used to test the independent contribution of probiotic use in predicting duration of hospitalization.

Results

A total of 60 patients with acute diarrhea secondary to rotavirus infection were included. Clinical and demographic characteristics of the study patients are summarized in Tables 1. Both groups had a similar number of infants ($p = 0.793$). No significant differences between groups were found with respect to age, gender, fever, grade of dehydration, ESR, CRP, blood leukocytes, but blood neutrophil counts were higher in control group ($p = 0.025$). A statistically significant difference in the duration of hospitalization was observed ($p < 0.001$) in children receiving probiotic (5.07 ± 1.30) in comparison to the control group (8.22 ± 2.14). Sub-analysis in infants showed the similar results (Table 2).

Regression analysis showed that probiotic use predict shorter of hospital stay ($\beta = 0.709$, $p < 0.001$, Table 3) independent of fever, grade of dehydration, ESR, CRP, blood leukocytes and neutrophil counts at admission day. No adverse effects in the probiotics group were observed by the investigators or nursing staff. All of the patients who concluded the study were followed-up after 48 h of discharge without signs of recurrence of the diarrhea.

Table 1. Clinical and demographic characteristics of the study patients

	Probiotic group	Control group	P Value
N	32	28	
Age (months)	17 (12-30)	17.5 (9.25-30.75)	0.843
Male gender (%)	20 (62.5%)	20 (71.4%)	
Temperature at admission	38.70±.61	38.61±.85	0.701،
Infants (%)	18 (64.3%)	10(35.7%)	0.793
Dehydration score			0.465
Mild <%5	11(34.4%)	6(21.4%)	
Moderate % 5–10	15(46.9%)	14(50.0%)	
Severe	6(18.8%)	8(28.6%)	
ESR	15.91±8.17	17.57±10.17	0.492
High CRP	16(50.0%)	12(42.9%)	0.613
WBC	8587.50±3443.16	7450.00±2734.48	0.160
Neutrophil (%)	0.52±.13	0.45±.14	0.061
Neutrophil count	4514.66±2569.74	3289.21±1441.93	0.025

Table 2. The duration of hospitalization days in study groups

	Probiotic group	Control group	P
Duration of hospitalization (Days)	5.07±1.30	8.22±2.14	<0.001
Infants	5.30±1.70	8.15±2.19	<0.001
Non-infants	4.94±1.06	8.26±2.16	0.002

Table 3. Results of regression analysis for predicting shorter of hospital stay

	Beta	P
Probiotic use	0.709	<0.001
Gender	-0.050	0.641
Age	0.038	0.724
Blood neutrophil count	-0.266	0.020
ESR	0.031	0.777
CRP	0.212	0.053
Grade of dehydration	-0.120	0.295
Temperature at admission	0.255	0.029

Discussion

In the present study probiotic preparations decreased the duration hospitalization in comparison to the control group, independent of fever, grade of dehydration, ESR, CRP, blood leukocytes and neutrophil counts at admission day. According to the natural history of the disease, it is well known that the duration of diarrhea secondary to rotavirus in non-malnourished children is around 5-7 days (14). The duration of treatment with probiotics is 5 days, hence in order to prevent any beneficial effects being falsely attributed to the treatment used, we included only patients with a history of diarrhea of less than 72 h before admission.

Probiotics have progressively gained in credibility for the treatment of in acute diarrhea and rotavirus diarrhea. A meta-analysis about probiotic therapy for acute diarrhea in children was associated with a significant reduction in duration of diarrhea especially in rotavirus gastroenteritis (5). Another meta-analysis, on the effect of several strains of lactobacilli in children with acute infectious diarrhea, revealed that probiotics reduced the duration and frequency of diarrhea (2). Several probiotic strains have been tested in hospitalized children with different results. Despite consistent evidence that probiotics reduce the duration of diarrhea, there is only weak evidence for their efficacy in reducing the duration of hospitalization(6).

Several studies in Iran, have evaluated probiotics in the treatment of infectious diarrhea in infants and in children with heterogonous results. The earliest study conducted by Kianifar et al. (10) on sixty-two Iranian children with acute non-bloody, non-bacterial diarrhea , showed that probiotic treatment consisting of a mixture of Lactobacillus acidophilus and bifidobacterium bifidum shortened the duration of diarrhea and hospital stay, and normalized stool frequency.

A similar study performed in 100 children with acute non- inflammatory gastroenteritis, the case group received probiotic yoghurt including lactobacillus bulgaricus, lactobacillus acidophilus, bifid bacterium, lactobacillus aciophilus and the control group received the ordinary yogurt. There were statistically significant differences between the case and control groups in reducing frequency of diarrhea in the first, second, third, and fourth therapy. Also, there was a significant difference in discontinuation of diarrhea between the case and control groups (9). In another randomized clinical trial on 80 children with non-bacterial gastroenteritis, consumption of probiotic yogurt reduces duration, severity and frequency of acute diarrhea and hospitalization days due to acute diarrhea (8). However, Abbaskhanian et al (7), showed that commercially fermented yogurt with 107 lactobacillus probiotics had no significant impact on minimizing any of symptoms in acute rotavirus diarrhea proven by stool using Enzyme - Linked ImmunoSorbent assay, and they conclude, importantly, our study emphasized the need for a cautious approach towards commercially probiotic fermented yogurt in the acute management of diarrheal disease in children. The test used to detect rotavirus in that was not ideal, resulting in the loss of patients for absence of disease or the presence of adenovirus, and introducing a bias risk in the studied patients.

Probiotics are a group of bacterial and fungal organisms thought to have beneficial effects on human health when they colonize the bowel. The delivery of nonpathogenic bacteria to the intestine is thought to provide protection via a number of mechanisms. First, antibacterial agents produced and secreted by such organisms, which include lactobacilli and bifidobacteria, may inhibit enteric pathogens(15). In addition, competition for mucosal receptor sites may inhibit adhesion and overgrowth of enterotoxic gram-negative aerobes (16) and enteropathogenic viruses (17) and permit more beneficial organisms to adhere to the surface. Higher fatty acid production and reduction of fecal pH may also show crucial roles in the inhibition of enterotoxic organisms (18). In addition, orally ingested Lactobacillus GG may also have immunomodulatory effects by an increase in the expression of mucin and IgA-secreting cells work-

ing against rotavirus, which may decrease intestinal inflammation. That probiotics may decreases the excretion of rotavirus during episodes of acute diarrhea, and probably this decreases the risk of transmission of rotavirus to healthy individuals, a fact that may exert a significant epidemiological impact (5, 19).

Conclusion

In summary, results of this study support the use of multi-strain preparation probiotics in treating rotavirus acute diarrhea.

Acknowledgements

This study was a part of Shaghayegh Ashraf - Talesh_final thesis for obtaining specialty of pediatrics from Iran University of Medical Sciences, Tehran, Iran.

Conflicts of interest: None declared.

References

1. Liu L, Johnson HL, Cousens S, Perin J, Scott S, Lawn JE, et al. Child Health Epidemiology Reference Group of WHO and UNICEF. Global, regional, and national causes of child mortality: an updated systematic analysis for 2010 with time trends since 2000. Lancet. 2014;379:2151–61.
2. Moradi-Lakeh M, Shakerian S, Yaghoubi M, Esteghamati A, Shokraneh F, Baradaran HR, et al. Rotavirus Infection in Children with Acute Gastroenteritis in Iran: A Systematic Review and Meta-analysis. Int J Prevent Med. 2014 5(10):1213–23.
3. Guarino A, Ashkenazi S, Gendrel D, Lo Vecchio A, Shamir R, H S. European Society for Pediatric Gastroenterology, Hepatology, and Nutrition/European Society for Pediatric Infectious Diseases evidence-based guidelines for the management of acute gastroenteritis in children in Europe: Update 2014. J Pediatr Gastroenterol Nutr. 2014;59(1):132-52.
4. Huang JS, Bousvaros A, Lee JW, Diaz A, EJ D. Efficacy of probiotic use in acute diarrhea in children: a meta-analysis. Dig Dis Sci. 2002;47(11):2625-34.
5. Ritchie ML, Romanuk TN. A meta-analysis of probiotic efficacy for gastrointestinal diseases. PLoS One. 2012 Apr 18;7(4):e34938.
6. Guarino A AS, Gendrel D, Lo Vecchio A, Shamir R, Szajewska H. European Society for Pediatric Gastroenterology, Hepatology, and Nutrition/European Society for Pediatric Infectious Diseases evidence-based guidelines for the management of acute gastroenteritis in children in Europe: update 2014. J Pediatr Gastroenterol Nutr. 2014;59(1):132-52.
7. Abbaskhanian Ali, Rezai MS, Karami H, A H. The effect of fermented yogurt on rotavirus diarrhea in children. Health Med. 2012; 6(5):1600.
8. Abedini M, Mortaz-Hejri G, Delpisheh A, Rasouli MA, A A. Effect of probiotic treatment on acute diarrhea in childhood. Journal of Food Safety and Hygiene. 2015;1(2):78-82.
9. Heydarian F, Kianifar HR, Ahanchian H, A K. A comparison between traditional yogurt and probiotic yogurt in non-inflammatory acute gastroenteritis. Saudi Med J.2010;31(3):280-3.
10. Kianifar HR, Farid R, Ahanchian H, Jabbari F, Moghiman T, A S. Probiotics in the treatment of acute diarrhea in young children. Iranian J Med Sci. 2009; 34(3):204-7.
11. MS K, MJG S. Fluid and Electrolyte Physiology and Therapy. In: Oski, F.A., Ed., Oski's Pediatrics: Principles and Practice. 4th Edition. Lippincott Williams & Wilkins, Philadelphia 2006, 54-70.
12. RG T. A Simple Concentration Method for the Detection of Parasitic Ova and Cysts in Faeces. J Clin Pathol. 1972;25:546-7.
13. Fortin J, MA P. Dehydration scoring system for infants. J Trop Pediatr Environ Child Health. 1978;24(3):110–4.
14. RF R. Pathogenesis of intestinal and systemic rotavirus infection. J Virol. 2004;78:10213—20.
15. Jack RW, Tagg JR, B R. Bacteriocins of gram-positive bacteria. Microbiol Rev. 1995;59:171–200.
16. Neeser JR, Granato D, Rouvet M, Servin A, Teneberg S, KA K. Lactobacillus johnsonii La1 shares carbohydrate-binding specificities with several enteropathogenic bacteria. Glycobiology. 2000; 10(11):1193–9.
17. Duffy LC, Zielezny MA, Riepenhoff-Talty M, Dryja D, Sayahtaheri-Altaie S, Griffiths E, et al. Effectiveness of Bifidobacterium bifidum in mediating the clinical course of murine rotavirus diarrhea. Pediatr Res. 1994;35(6):690–5.
18. Ohya T, Marubashi T, H I. Significance of fecal volatile fatty acids in shedding of Escherichia coli 0157 from calves: experimental infection and preliminary use of a probiotic product. J Vet Med Sci. 2000; 62(11):1151–5.
19. Sakamoto I, Igarashi M, Kimura K, Takagi A, Miwa T, Y K. Suppressive effect of Lactobacillus gasseri OLL 2716(LG21) on Helicobacter pylori infection in humans. J Antimicrob Chemother. 2001;47:709–10.