

***Lactobacillus acidophilus* Yogurt and Supplement in Children with Acute Diarrhea: A Clinical Trial**

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Abstract: Acute gastroenteritis is a major cause of children morbidity and mortality worldwide. Probiotics like *Lactobacillus Acidophilus* (*L.acidophilus*) may be beneficial in treatment of acute diarrhea. This study aimed at comparing the effects of yogurt fermented by *L.acidophilus* (*L.acidophilus* yogurt), *L.acidophilus* supplement, conventional yogurt and placebo added to routine fluid therapy in children with acute non-bloody diarrhea. In a randomized clinical trial, 160 children with acute diarrhea admitted in Tabriz Children Hospital were assessed in a clinical trial. They were randomized in 4 groups: Group A received 300 g day⁻¹ of yogurt fermented by lactobacillus acidophilus, group B received 2 capsules/day of lactobacillus acidophilus supplement, group C received 300 g day⁻¹ of conventional yogurt and group D received placebo material (control group). The standard fluid therapy was administered in all patients. Age, sex, duration of diarrhea before admission, weigh on admission and discharge and its change, frequency of stool passing on days 1, 2 and 3 post-admission and their changes, type of diarrhea, signs and symptoms on admission and day 3 and duration of hospital stay were determined in each patient. One hundred (62.5%) males and 60 (37.5%) females, with the mean age of 18.36±6.47 (12-48) months enrolled in the study. Four groups were matched considering the baseline characteristics. The mean frequency of stool passing on day 3 post-admission and the mean amount of decrement in stool passing frequency 72 h after hospitalization were significantly higher and lower in group A, respectively (p = 0.015 and 0.029, respectively). Dehydration rate on day 3 was significantly lower in groups A and B (p<0.001). The mean duration of hospital stay was significantly shorter in group B (p=0.037). *L.acidophilus* yogurt and *L.acidophilus* supplement would diminish the severity of acute non-bloody diarrhea in children.

Key words: Acute diarrhea, *Lactobacillus acidophilus*, yogurt, supplement, children morbidity

INTRODUCTION

Diarrhea is common among children and contributes substantially to pediatric morbidity and mortality world wide (Van Niel *et al.*, 2002). In developing countries, an estimated overall incidence of acute gastroenteritis ranges from 6-12 episodes of diarrhea per year in children under 5 years of age compared to 1.3-2.3 episodes in developed countries. In developing countries, approximately 2.4-3.3 million children below 5 years of age die per year from diarrhea (Glass *et al.*, 1991). Given the ubiquity of acute diarrhea and its associated burdens on children, families and the health care system, all parties desire a therapy that is safe and relatively inexpensive. For at least a century, researchers have hypothesized that live bacterial

cultures, such as those found in yogurt, may help treat and prevent diarrhea (Metchnikoff *et al.*, 1901). The bacterial genus *Lactobacillus*, which is found in normal human intestinal and perineal flora, has been studied frequently in children with regard to its antidiarrheal properties since the 1960s (Camatte, 1966; Pene *et al.*, 1966). These materials are generally termed as probiotics. Probiotics have been defined as living micro-organisms that, upon ingestion in certain numbers, exert healthbenefits beyond general nutrition (Van Niel *et al.*, 2002). Probiotic functionality depends on the ability of a strain to confer health advantages on a host upon oral consumption of viable cells (Sanders and Klaenhammer, 2001). Studies published in the world literature have concluded that *Lactobacillus* is indeed safe and effective

in treating and preventing infected diarrhea; antibiotic-associated diarrhea and diarrhea in children who are unusually susceptible as a result of poor nutrition, impaired immune status, or frequent exposure to pathogens (Van Niel *et al.*, 2002). Many different strains and species of lactobacilli have been used commercially as probiotics. Numerous mechanisms have been proposed for probiotic functionality and many clinical end targets have been measured. The preservation of the integrity of the normal intestinal flora, colonization resistance, adherence and production of antibacterial substances appear to be important for these effects. Although the precise mechanisms of action are still unclear, yogurt consumption has increased significantly in recent years, presumably in part because of these perceived health benefits (Trapp *et al.*, 1993). It is difficult to assess the health effects that might be expected from probiotic consumption by the general population, since most research to date has focused on animal studies, biomarkers, or small human study group sizes. More extensive epidemiological evaluations will be necessary to better study these properties (Sanders and Klaenhammer, 1991). Conventional yogurt is fermented milk produced by the addition of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* to milk. Based on increasing evidence that other lactic acid bacteria such as *Lactobacillus acidophilus* (*L.acidophilus*) has therapeutic properties, this species has also caught the eye of researchers. Although conventional yogurt bacteria have a very poor intrinsic resistance to acid and bile, *L.acidophilus* can tolerate a pH 3 and 2-8% concentrations of bile acid (Marteau and Rambaud, 1993; Gilliland, 1989).

This study aimed at evaluating the outcome of child and infant patients with acute non-bloody diarrhea receive yogurt with *L.acidophilus* starter (*L.acidophilus* yogurt), *L.acidophilus* supplement, conventional yogurt and placebo along with routine fluid therapy and comparing them.

MATERIALS AND METHODS

In a randomized clinical trial, one hundred sixty patients out of 178 children with acute non-bloody diarrhea admitted in Tabriz Children Hospital during one year (May2005- May2006) were enrolled in this study. The patients met the following criteria:

A history of three or more watery stool per day for less than 48 h, b-no bloody stools at the first examination, c-clinical signs of dehydration, d-no evidence of hypovolemic shock, e-no evidence of co-existing acute systemic illness and f-no history of current antibiotic or anti diarrhea medication use.

They randomized into 460 patient groups; first group received *L.acidophilus* yogurt (300 g day⁻¹, semi-fluid), second group was given 2 capsules per day (made by Tabriz Drug Research Center, containing 5×10⁹ Colony-Forming Units (CFU) g⁻¹ of *L.acidophilus*), third group received conventional yogurt (300 g day⁻¹, semi-fluid) and the forth group received placebo material. Each child admitted into the study was allocated to either treatment group by restricted randomization using random permuted blocks. The degree of dehydration was assessed clinically. All the patients treated by fluid therapy in accordance with the WHO's guidelines (World Health Organization, 1990). Children were not given other fluid or foods during the study period. All the patients were hospitalized and supervised with skilled staff. Feeding was carried out by these personnel, as well. Informed consent from the parents was obtained and demographic and clinical data were registered. Age, sex, duration of diarrhea before admission, weigh on admission and discharge and its change, frequency of stool passing on days 1, 2 and 3 post-admission and their changes, type of diarrhea, signs and symptoms on admission and day 3 and duration of hospital stay were determined in each patient. The patients were discharged from the hospital 24 h after cessation of diarrhea. The study protocol was approved by the ethical and research review committees of Tabriz medical university.

Statistical analysis: Data were analyzed with the SPSS statistical software package (version 15.0; SPSS Inc., Chicago). Continuous variables were expressed as mean± standard deviation and categorical data were shown as frequency and percent. The contingency table (The Chi square and The Fisher's exact tests where appropriate) and the Independent samples T test or One Way ANOVA test employed for comparisons. P values below 0.05 were considered statistically significant.

RESULTS

A total of 178 patients with non-bloody acute diarrhea admitted to hospital during the study period. Eighteen patients were withdrawn from the study, mainly due to parental non-compliance. Finally, 160 remaining patients were enrolled in the study and randomized in 4 groups (40 patients in each one). The presenting signs and symptoms are shown in Fig. 1. There was no patient with either positive stool exam or culture. No parasitic cause was defined either. Characteristics of the patients and the main outcomes are summarized in Table 1. The patients of the four groups were entirely matched regarding the age, sex and the condition of diarrhea. As shown, the mean frequency of stool passing on day 3

Table 1: Characteristics of the patients and the main results

Group	<i>L.acidophilus</i> yogurt (n=40)	<i>L.acidophilus</i> supplement (n=40)	Conventional yogurt (n=40)	Control (n=40)	p
Gender					
Male	27	27	23	23	NS
Female	13	13	17	17	
Age (month)	17.6±5.5	19.6±7.1	19.0±7.8	17.3±5.0	NS
Duration of diarrhea before admission (day)	1.0±0.1	1.6±0.2	1.4±0.5	1.2±0.0	NS
Type of diarrhea					
Watery	38	37	40	39	NS
Mucoid	2	3	-	1	
Weight on admission (kg)	10.2±1.4	10.8±2.0	10.5±1.7	10.6±1.8	NS
Weight at discharge (kg)	9.9±1.4	10.7±2.0	10.1±1.7	10.4±1.9	NS
Weight change (discharge-admission)	0.3±0.5	0.2±0.6	0.3±0.7	0.2±0.6	NS
Dehydration on admission					
Mild	2	2	3	3	NS
Moderate	36	37	37	35	
Severe	2	1	-	2	
Stool frequency in day 1	5.5±3.6	3.7±3.0	5.2±4.8	3.8±3.3	NS
Stool frequency in day 2	3.7±3.5	4.0±3.2	5.8±4.9	4.0±3.6	NS
Stool frequency in day 3	1.3±2.0	1.4±2.6	3.3±4.3	2.3±2.6	0.015
Change of stool frequency (day 1-day 2)	1.8±3.7	-0.3±3.9	-0.7±6.0	-0.3±4.5	NS
Change of stool frequency (day 1-day 3)	4.2±3.8	2.2±3.7	1.9±5.6	1.5±3.8	0.029
Change of stool frequency (day 2-day 3)	2.4±2.5	2.6±2.6	2.6±3.3	1.8±2.9	NS
Dehydration (day 3)	-	-	7	12	<0.001
Hospital stay (day)	3.6±0.9	3.4±0.9	3.9±1.2	4.0±1.1	0.037

L.acidophilus: Lactobacillus Acidophilus, NS: Not Significant, p=0.05: Significant

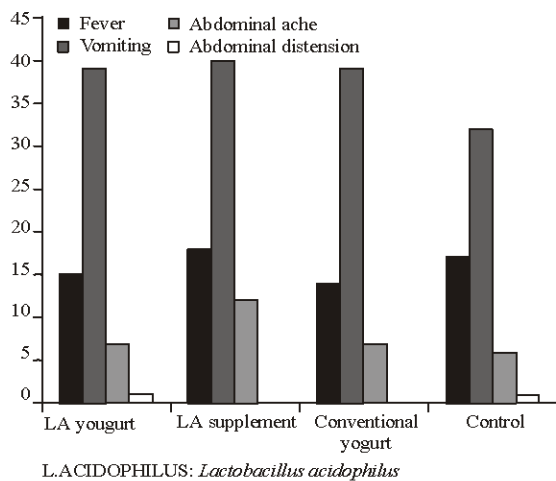


Fig. 1: Signs and symptoms on admission

post-admission was significantly lower in patients received *L.acidophilus* yogurt. The mean decrement of stool passing frequency on day 3 in comparison with the same value on admission day was significantly greater in the same group, as well. On day 3 post-admission, there were still 7 and 12 cases with moderate dehydration in groups given conventional yogurt or placebo, respectively. Dehydration was significantly more frequent in the control group. The mean duration of hospital stay was significantly shorter in patients received *L.acidophilus* supplement. The sign and symptoms of the patients on day 3 post-admission are shown in Fig. 2. Apparently they are more frequent

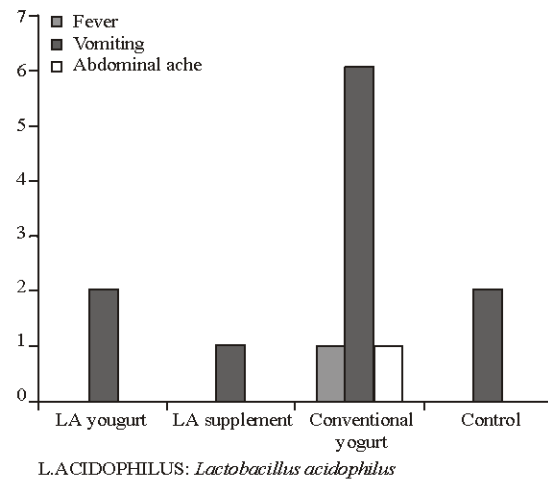


Fig. 2: Signs and symptoms on day 3 post-admission

(vomiting in particular) in group supplemented by conventional yogurt; however, statistical confirmation was not applicable.

Comparison with the control group

***L.acidophilus* yogurt group:** Dehydration frequency on day 3 post-admission was significantly higher in the control group ($p<0.001$). The mean decrease of stool passing frequency in days 2 and 3 post-admission comparing with the first day was significantly more in *L.acidophilus* yogurt group ($p=0.028$ and 0.002 , respectively). Other parameters shown in Table 1 were not significantly different between the two groups.

***L.acidophilus* supplement group:** Dehydration frequency on day 3 post-admission was significantly higher in the control group ($p < 0.001$). Hospital stay was significantly shorter in *L.acidophilus* supplement group ($p = 0.030$). Other parameters shown in Table 1 were not significantly different between the two groups.

Conventional yogurt group: None of the parameters shown in Table 1 were significantly different between the two groups.

DISCUSSION

In the current study, we tried to compare the outcome of 4 groups of children with acute non-bloody diarrhea received placebo, conventional yogurt, yogurt with lactobacillus acidophilus (*L.acidophilus*) starter and *L.acidophilus* supplement as well as the conventional therapies. For a better result, all the studied cases were hospitalized and were given care and monitored by skilled hospital staff. So any probable error due to parents' incompetence has been minimized. Likewise, the comparison of baseline characteristics showed no significant differences between groups. Adding the *L.acidophilus* yogurt to the routine therapy of acute diarrhea in children significantly decreased the frequency of watery stool passing and the rate of dehydration during hospitalization comparing with the control group. Tejada-Simon *et al.* (1999) showed that administration of yogurt supplemented with *L.acidophilus* and Bifidobacterium species enhanced mucosal and systemic IgA responses to the cholera toxin immunogen; hence producing a better protecting response to diarrhea. Kaila *et al.* (1992) carried out a study about supplementary effect of Lactobacillus GG (a similar species to *L.acidophilus*) fermented milk in acute diarrhea. The results indicate that Lactobacillus GG promotes recovery from rotavirus diarrhea (one of the most common causes of acute non-bloody diarrhea in children and infants) via augmentation of the local immune defense. Furthermore, specific IgA response to rotavirus is endorsed, which is possibly relevant in protection against reinfections. In our study, patients who received a *L.acidophilus* supplement (capsule) also showed a lower incidence of dehydration during hospital stay. Furthermore, hospital stay (i.e. the duration of diarrhea) was significantly shorter in this group comparing with the placebo takers. Lee *et al.* (2001) concluded that oral bacterial therapy with *L.acidophilus* is an effective adjuvant therapy in rotavirus positive and negative children with diarrhea and can safely be administered during an episode of acute diarrhea. The duration of

diarrhea during hospitalization in study group decreased, as well. Many other studies (Boulloche *et al.*, 1994; Simakachorn *et al.*, 2000; Guandalini *et al.*, 2000; Shornikova *et al.*, 1997; Raza *et al.*, 1995; Pearce and Hamilton, 1974; Chicoine and Joncas, 1973) have ever reported a beneficial effect of *L.acidophilus* supplements on acute diarrhea; however, as our results, Khanna *et al.* (2005) did not report a preponderance for tyndalized *L.acidophilus* over placebo in diminishing the frequency of stool passing in acute diarrhea. Some studies (Pashapour, 2006; Gorbach, 1990; Boudraa *et al.*, 2001; Beniwal *et al.*, 2003) previously showed that the conventional yogurt may improve the healing process of acute diarrhea. In our study, in contrast, adding a diet of conventional yogurt did not improved the condition of patients with acute diarrhea comparing with the control group. Difference in the starter type, the amount of administration and the way of assessment may lead in heterogeneous consequences (Sanders and Klaenhammer, 2001). Further studies with more controlled parameters may yield a more reliable out-turn. To our knowledge, no other study has been yet done comparing the mentioned 4 groups. We revealed that the *L.acidophilus* yogurt is the most superior supplement for diminishing the frequency of watery or mucoid stool passing in children with acute non-bloody diarrhea. On the other hand, the duration of diarrhea was significantly shorter in patients received *L.acidophilus* supplement in combination with routine fluid therapy. Both supplements have been accompanied with a lower incidence of dehydration comparing with the conventional yogurt or placebo groups. There is evidence (Perdigon *et al.*, 1985, 1986, 1987; Solis Pereyra and Lemonnier, 1993) that ingestion of lactic acid bacteria and the related food products exerts an immunomodulatory effect in the gastrointestinal system of both humans and animals. The gastrointestinal system possesses specialized elements that react upon exposure to antigens coming from diet and that result in immune reactions (Pestka, 1993). Current study confirmed the previous reports in this regard. Adding the *L.acidophilus* fermented milk, as a safe, inexpensive, available and easily acceptable product to the routine strategy of treatment in children with acute non-bloody diarrhea makes sense, since the potential morbidity and mortality of the disease, as well as the huge costs loading on the health system shoulder could at least be decreased. Using the commercially prepared supplements in this regard, have some extra benefits such as a dose-determined route of administration and a healthier product with lesser contamination; however the price would be raised. According to this study, for an optimal result with both diminishing the frequency of

stool passing and shortening the duration of diarrhea and hospital stay, combination therapy with *L.acidophilus* fermented yogurt, oral *L.acidophilus* preparations and the proper liquid therapy according to accepted guidelines is recommended in these children.

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