EVALUATION OF THE ROLE OF LACTOBACILLUS GG IN THE MANAGEMENT OF ACUTE DIARRHOEA AT KANTI CHILDREN'S HOSPITAL, KATHMANDU

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KATHMANDU
NEPAL .

THE KANTI CHILDREN'S HOSPITAL (1998)

	Beds
Surgical	36
SICU	4
NICU	14
PICU	6
Observation	10
Burn Unit	16
Medical	56
General	40
Special Cabin	18
VIP Cabin	6
Cabin	14
ORT / ARI	30
Total	250

Source :- Nepal Journal , March 1998

Diarrhoea ward	20	beds
Annual admission	4530	(1997)

Dehydration

No	1773
Some	1987
Severe	770

Age groups

0 ____ 1 year	1280
1 ____ 5 year	1624
5 ____ 10 year	1313
10 ____ 15 year	313
Total	4530

Source :- Diarrhoea ward Kanti Children's Hospital (1998)

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Abbreviations

ATCC 53103	---	American Type Culture Collection with the accession number 53103
CFU	---	Colony Forming units
CI	---	Confidence Interval
CO_2	---	Carbon dioxide
GIT	---	Gastro Intestinal tract
IV	---	Intravenous
J	---	Journal
LGG	---	Lactobacillus GG
LSTM	---	Liverpool School of Tropical Medicine
NCHS	---	National Center for Health Statistics (US)
ORS	---	Oral Rehydration Solution
ORT	---	Oral Rehydration Theray
RB-ORS	---	Rice Based Oral Rehydration Solution
SD	--	Standard Deviation
UTI	---	Urinary Tract Infection
WHO	---	World Health Organization

Glossary

Lactobacillus GG	--	Named after the discoverers, Gorbach and Goldin, and now identified as Lactobacillus casei subspecies rhamnosus or L. rhamnosus .
Probiotics	--	A live microbial food supplement that beneficially affects the host animal by improving the microbial balance . Probiotics are used in fermented dairy products and cheeses .
Bifidobacterium	--	A genus of anaerobic bacteria (family Actinomycetaceae) containing gram-positive rods of highly variable appearance. Freshly isolated strains characteristically show bifurcated V and Y forms, uniform or branched, and club or spatulate forms. Bifidobacteria appear in the alimentary tract and faeces of infants and other people and in other animals .

Objectives

Main objective :-

The main objective of this study was to assess the effectiveness of early refeeding supplemented with Lactobacillus GG 10^{10} cfu twice daily in the management of acute diarrhoea .

Specific objectives :-

1. To assess the effectiveness of Lactobacillus GG in reducing the duration of diarrhoea and length of hospital stay .
2. To assess the effectiveness of Lactobacillus GG in reducing the stool frequency .
3. To identify the microorganisms causing diarrhoea in the study group .

Abstract

To determine the role of Lactobacillus casei strain GG in the management of acute diarrhoea (mixed causes some & severe dehydration), a prospective, placebo controlled, triple blind clinical trial was undertaken in Kanti Children's Hospital, Kathmandu . One hundred children between one month and 24 months were enrolled in the study over a period of 1st July to 24th September 1997 . After oral rehydration for 6 hours, the study cases randomly received either Lactobacillus GG freeze dried powder 10^{10-11} colony forming units twice daily for three days (n = 50) or a placebo (n = 50), in addition to the usual diet & standard antibiotic treatment based on WHO guidelines .

There was no significant difference in reducing the duration of diarrhoea and frequency of stool on the third day of treatment in those receiving Lactobacillus GG compared to the placebo group . Further studies of the effectiveness of the use of Lactobacillus GG in developing countries in the management of gastroenteritis are required, and should involve a more prolonged period of follow up in different seasons of the year .

Introduction

Acute diarrhoeal disease is one of the leading causes of childhood mortality and morbidity in the developing countries, and a major contributor to malnutrition . Diarrhoea leads to dehydration regardless of aetiology . Dehydration is the major cause of death . The widespread use of oral rehydration solution (ORS) has been important in reducing the risk of dehydration, but it neither shortens duration of diarrhoea nor provide any significant nutritional value . There has been a recent shift in emphasis in the nutritional strategies used to promote recovery from diarrhoea . Following rehydration, early feeding with mixed milk feeds, yoghurt or traditional fermented foods have been shown to improve outcome as well as to provide nutrients (28, 29, 30). The continuation of breast feeding remains important (31) .

Another approach to promote recovery, which partly explains the effect of fermented feeds, is to colonize the gut with non-pathogenic bacteria that may replace the pathogens . Lactobacillus casei strain GG (Lactobacillus GG) is a human isolate of Lactobacillus that can colonize the bowel, and demonstrates both antimicrobial and Immuno-stimulatory activity in the gut . A study of Finnish children with gastroenteritis, predominantly due to rotavirus, suggested that Lactobacillus GG had a significant effect in shortening the course of acute diarrhoea (17).Moreover further two studies in the tropical countries has shown the beneficial effect of LGG in watery diarrhoea (14, 41) .

Lactobacillus

Lactobacillus (L) are non pathogenic organisms that have been used in food from ancient times and are normal constituent of gastrointestinal tract of human beings. Lactobacillus which are used as probiotics i.e. products containing health promoting bacteria mainly in dairy food and in agriculture industry are as follows (1)

1. L. acidophilus - as a supplement in dairy products and used for fermentation.
2. L. plantarum - in dairy products, pickled vegetables and silage.
3. L. casei species GG in yoghurt and whey drink.
4. L. casei subspecies rhamnosus - in dairy product and silage.
5. L. brevis - in dairy products and silage.
6. L. delbrueckii sub-sp. (bulgaricus) for the production of yoghurt.

There is however, host specificity in colonization by individual species eg. L. acidophilus, L. fermentum, L. plantarum and L. casei (GG) are commonly found in the faeces of humans. Whereas L. bulgaricus used, to make yoghurt, is unable to colonize the gastrointestinal tract (g.i.t) and is not isolated in the faeces (2) . The reason for this specificity has not been described, although, animal studies have suggested that Lactobacilli colonize mucosal surfaces of the gut , vagina and urethra by binding epithelial cells. (3, 4, 5)

Numerous health claims have been made for a number of species (e.g L. acidophilus, L. casei GG or L. bulgaricus) not all of which have been substantiated.

In a number of different ways, Lactobacillus may be beneficial by altering the intestinal microflora. The nutritional effects include increased nutritional utilization and alleviation of lactose intolerance. The therapeutic effects include uses in the treatment of hepatic enephalopathy, gastrointestinal infection and the inhibition of carcinogens in the g.i.t (6)

Lactobacillus GG

Gorbach and Goldin in 1983 isolated a strain of Lactobacillus, which is able to colonize the human intestinal tract and adhers more tightly to human intestinal and buccal cells than other strains of Lactobacillus. It was named as Lactobacillus GG (L G G) in 1985. This strain was deposited in the American Types culture collection, with the accession number 53103 (ATCC-53103) Moreover it has ability to resist acid & bile, produce antimicrobial substance, grow well & shows beneficial effect on human health. (7)

Gorbach in 1990 has also demonstrated that when a LGG in a lyophilized form or as a fermented milk product is consumed by an individual it is consistently in faeces in concentration detected > 10^{6-1}g. The Lactobacillus GG strain elaborates an antimicrobial substance which has a broad spectrum of activity against a range of bacteria, including clostridium difficile (8) . Moreover colonies have a unique morphology when cultured on MRS agar which facilitates their identification in the stool. LGG grow better under anaerobic condition, although it can grow aerobically in the presence of carbon dioxide (co_2). The electrophoretic pattern of soluble protein of LGG most closely resemble that of L. casei subspecies rhmnosus, but Lactobacillus GG differs from that species in that it does not ferment lactose, maltose or sucrose. The strain produces small amount of acetic acid and large amounts of lactic acid. LGG is sensitive to penicillin, clindamycin, erythromycin, vancomycin, tetracycline and chloramphenicol, but it is resistant to metronidazole. LGG when grown on LBS- tomato juice agar and MRS agar looks like a large, creamy white colony that emits a buttery oder (9)

LGG when gram stained from colonies look like large, uniform, gram positive rods mainly in chains. It is distinguished from most other Lactobacilli by its relative inability to ferment lactose (10). Lactobacillus bulgaricus is the most susceptible to acid (PH^3) (9).

Goldin et al, have shown the longer persistence of LGG than E. Coli in human g.i.t in 81% after 4 days and in 33% after 7 days of discontinuation of treatment (9). They have also been able to show the decrease in faecal B-glucuronidase activity by LGG. B-glucuronidase catalyses the hydrolysis of conjugates of glucoronic acid.

These conjugates are formed in the liver to overcome their solubility in water. Depending on the structure of the glucuronidase, it can be excreted in the bile, followed by intestinal deconjugation, which can result in generation of active drugs, hormones & proximal carcinogens (11). Intestinal B-glucuronidase is also altered by diet & antibiotics. The mechanism by which the LGG alters B-glucuronidase activity is unknown. It is possible that LGG by lowering the numbers of a selective group of bacteria via its known antimicrobial activity (10), can grow B-glucuronidase activity without drastic changes in the composition of the intestinal microflora.

Various types of Lactobacillus have been shown to exert a beneficial effect in conditions such as Diarrhoea, Vaginal pruritus, U.T.I. and experimental colon cancer (8). Moreover, it has been found beneficial in lowering the incidence of travellers diarrhoea in one group of Europeans travelling to Turkey. These people had a reduced incidence of travellers diarrhoea to the order of 40% (12).

LGG (ATCC 53103) is one of the most extensively studied probiotic lactic-acid bacteria strains. The safety assessment of LGG covers traditional toxicity tests and studies on the safety of strain in invivo and invitro condition. A number of studies in different clinical conditions, human volunteer studies and epidemiologic surveillance indicate that the strain is safe for human consumption even in large amounts. At present LGG has the most extensive safety assessment record when compared with other probiotic strains (13). Another trail of LGG was conducted by Pant in the Bangkok children's Hospital (14), he studied 39 children with Acute diarrhoea who were an average of 8 months. Following rehydration received either oral LGG (N=20) as a freeze dried preparation or placebo (n = 19) twice daily for two days. When only those with acute non bloody diarrhoea (n = 26) were considered, the mean duration of diarrhoea was significantly shorter in the LGG group (1.9 days) than in placebo group (3.3 days) ($P<0.05$). Stool frequency was less on the second day in the Lactobacillus group ($P<0.05$). The results indicate that LGG accelerates recovery from acute watery diarrhoea in a tropical setting.

Advantages of Lactobacillus GG

Colonization of G.I.T and changes in Faecal enzymes after oral Lactobacillus GG administration

Colonization of g.i.t. is an important property for a beneficial effect of Lactobacillus. Salminen et al in 1991 (16) in their study (adult volunteers 18-55 years) observed colonization of faeces after oral doses from and above 10^9 cfu /day. The dose level was from 1.5×10^6 to 1.1×10^{11} cfu / day for four weeks. A dose of 10^{10} cfu / day colonized all volunteers. However, one volunteer was colonized only on days 2, 3 and 4 of treatment. During the study period, the mean level of Lactobacillus GG in faecal samples was 10^5 to 10^6 cfu / g faeces. Moreover, with the increase in dose (e.g 11^{11} cfu) Lactobacillus did not appear to influence the total number of lactic acid bacteria in faeces.

B-glucuronidase levels were slightly decreased during the seventh day of Lactobacillus GG treatment. However, after four weeks of treatment with 10^{10} cfu / day of Lactobacillus GG, B- glucuronidase levels were decreased by 80% .

Infantile Diarrhoea and Lactobacillus GG

Some studies have demonstrated the beneficial effect of preparations containing Lactic acid bacteria or fermented dairy products on recovery of infants from gastroenteritis : Isolauri et al 1991 (17) , has been successful in demonstrating a beneficial effect of Lactobacillus GG in reducing the hospital duration of diarrhoea. In their study, Lactobacillus GG was investigated for the treatment of infants with diarrhoea, mainly due to rotavirus. After oral rehydration, the hospitalised infants aged between 4-45 months were randomly divided into three groups . Group 1, received a Lactobacillus GG fermented milk product (10^{10} -10^{11} cfu) . Group 2, received the same number of organisms as a freeze dried powder and group 3, received yoghurt containing no live Lactic acid bacteria. All treatments were given twice daily for a period of 5 days in addition to normal diet which was free of other fermented dairy products . The mean (± S D) number of days of diarrhoea after commencing therapy was significantly shorter in group 1 (1.4 ± 0.8 days) and in group 2 (1.4 ± 0.8 days) than in group 3 (2.4 ± 1.1 days) $F = 8.70$ ($P < 0.001$) . These results suggest that fermented dairy products with viable Lactic acid bacteria that are able to colonize the gut, may be effective in shortening the course of acute diarrhoea.

These finding were supported by another study (18) in which 45 infants hospitalised for diarrhoea were given either yoghurt as treatment or a neomycin / kaolin / pectin mixture. The duration of disease was significantly less in the yoghurt group compared with the other treatment group.

Lactose intolerance and Lactobacillus

Lactose (a milk carbohydrate, disaccharide) causes intestinal distress when consumed by people with a deficiency in the intestinal mucosal enzyme B-galactosidase (lactose) . Such individuals must restrict their intake of milk and dairy products. During fermentation, Lactobacilli produce lactase which hydrolyses milk lactose to glucose and galactase. The production of lactase increases steadily during yoghurt fermentation . Lactase can also be released from bacterial cultures during digestion indicating that bacterial lactase is present in the intestine of persons consuming yoghurt (19) .

Lactose intolerant subjects reported abdominal distress when drinking milk, but they did not report any such problems when given yoghurt (20) . Kolars et at in 1984 (21) had used the breath hydrogen measurement to demonstrate that lactose intolerant subects given 18 g of lactose in yoghurt had only about one third as much hydrogen excretion as those given a similar amount of lactose in milk and water . Analysis of duodenal aspirates indicated significant lactose activity in the intestine 1 hour after ingestion of yoghurt .

Antitumour activity and Lactobacillus

Fermented dairy products or starter cultures used by the dairy industry (antitumour effect of L. acidophilus) have been shown to either inhibit chemically induced colon tumours or transplantable tumour lines in rodents (22, 23) . The bacterial enzymes that were affected included B-glucuronidase, azoreductase and nitroreductase. These enzymes catalyse the conversion of procarcinogens to proximal carcinogens in the large bowel.

Hepatic Encephalopathy and Lactobacillus

Hepatic encephalopathy is a neurologic disorder associated with liver failure and elevated blood ammonia levels . Ammonia is normally produced in the intestine from urea by the action of bacterial urease . The ammonia is absorbed and detoxified in the liver . In patients suffering from liver failure the detoxification mechanism is impaired and the ammonia levels rise in the circulating blood . Treatment with L. acidophilus and / or neomycin results in a lowering of faecal urease and a decrease in ammonia levels of patients with hepatic encephalopathy (24) . These metabolic effects are associated with improvement in the clinical status of the patient .

Antimicrobial activity of Lactobacillus

The antimicrobial substance produced by Lactobacillus GG can control the proliferation of a wide range of gram negative and gram positive organisms . This antimicrobial substance shows, however no inhibitory activity against other Lactobacilli . Lactobacillus GG has been found to be useful in the treatment of relapsing clostridium difficile colitis (26) and in the prevention of traveller's diarrhoea .

Immunostimulation by Lactobacilli

Perdigon et al (25) have demonstrated the immunostimulation effect when fed with milk fermented with L. casei or L. acidophilus by the activation of macrophages and lymphocytes . This finding has been supported by another study by kaila et al (1992) . In their study of 44 well nourished children aged 7 and 37 months of age, 39 (88%) patient were rotavirus positive . They formed the actual study (n = 22) and placebo (n = 17) groups . The patients were rehydrated and realimented . The duration of diarrhoea was significantly reduced in the study group 1.1 (0.6) days compared to the placebo group 2.5 (1.4) days (P = 0.001) (27) .

A prominent Ig - secreting cell was measured during the acute phase of diarrhoea in both groups, especially in cells secreting Ig M. The transient response in the study group was almost twice that in the placebo group in all Ig classes . But at the convalescent phase, the difference between the two groups was no longer seen .Patient in both the study group and the placebo group showed a similar specific antibody secreting (SASC) response to rotavirus in Ig M class [95 (95% CI 46, 198) and 82 (95% CI, 200) SASC / 10^6 cells, respectively P = 0.79] . Three weeks later, the numbers were low in both groups .

Methods

The study was conducted in the diarrhoea ward of Kanti Children's Hospital, Kathmandu over 12 weeks period between 1st July & 24th September 1997. This period was the rainy season, & which was the peak season for acute diarrhoea in Kathmandu. A sample size of 100 (50 in each group) was calculated with the help of epidemiologist, at the School of Tropical Medicine, Liverpool, U.K. The study subject included children aged 2 mo and 24 mo, consecutively admitted with acute diarrhoea (diarrhoea of less than 14 days duration and with more than three watery stools during the previous 24 hrs). The admission number in the study group was controlled to maximum three per day.

Randomization of placebo and drugs was carried out by Maija Saxelin, Valio Ltd, Helsinki, Finland. Moreover randomized drugs / placebo were obtained in a sealed plastic bottle labelled drugs for treatment of acute diarrhoea with a number in each bottle.

Children below 1 and over 24 months & septicaemic were excluded from the study. The study project was approved by Nepal Health Research Council, Ramshah Path, Kathmandu and permission to conduct the study was given by the Director of Kanti Children's Hospital, Kathmandu. Consent was taken from parents in each case. On admission, each patients was weighed & examined clinically assessing degree of dehydration according to a standard WHO protocol (15). Those with some dehydration were given oral rehydration while those with severe dehydration received intravenous fluid therapy, according to the usual protocol of Kanti children's Hospital. After the initial rehydration period (6 hours), children received either Lactobacillus GG (10^{10}-10^{11} colony forming units) as a freeze-dried preparation or a placebo mixed in 10 mls of ORS and given orally. Both Lactobacillus GG and placebo had the same appearance & texture. No problems were encountered with either preparation on the ward or with administering the treatment to the child. Treatment was continued at 12 hourly intervals for 3 days (i.e 6 doses), in addition to the usual diet. Both exclusive breast feeding below 6 month & mixed milk diet, which was the usual practice were included in the study. Standard antibiotic treatment as per WHO guidelines was given for cholera, shigella, & E. histolytica .

Data Collection

Data collected included age, sex, duration and frequency of both diarrhoea & vomiting, character of the stool (classified as watery with or without blood) and any treatment prior to admission, including antibiotics or antidiarrhoeal medicine. Weight and axillary temperature were recorded daily.

Fluid intake, episodes of vomiting, frequency of diarrhoea and character of stool (watery, loose or formed were recorded over the next 72 hours.

Each child was followed until discharge, and duration of diarrhoea was recorded as the time from admission until the time of the last watery stool. It was not possible to follow the patients beyond hospital discharge.

Stool samples collected on admission & at 72 hours were examined for ova, cysts, parasites, reducing substances and cultures. A portion of each sample was stored in an ependrof at 4^0c and transported to Liverpool, U.K., at the end of the study in a sealed ice pack for virological analysis at the Department of Medical Microbiology, University of Liverpool, U. K.

Statistics

The statistical analysis was carried out blindly using EPI-INFO programme and the code was only broken once all the results and tables had been prepared. It was carried out at the Institute of Medicine, Kathmandu, by an senior statistician .

Results

A total of 100 children (1 month to 24 months) were enrolled in the study . Fifty children received Lactobacillus and another fifty received the placebo. The patient characteristics were similar for each group and are summarized in the table 1 .

Results of bacteriological analysis of stool samples (direct microscopy and culture of stool & special staining if needed) are shown in the table .

Reducing substance (sugar ≥ 0.5 mg%) was positive in 37% . A virus was identified in 29 cases (29%), using Electron Microscopy. Among which Rotavirus was positive in 9%, Adenovirus in 10%, Enterovirus in 2% & Corona virus in 8% of cases . (Table)

Outcome of therapy

Following successful rehydration, all patients were managed according to their treatment protocol based on recent WHO guidelines . There was no difficulty in mixing the drugs / placebo & compliance was good in both the groups . None of the patient vomited in either group . The duration of diarrhoea (days) was less in Lactobacillus group but was not statistically significant .

The recovery was more faster in LGG group, in terms of frequency but the difference was not statistically significant .

Table 1 : Characteristics of patients on admission

Characteristic	Placebo Group n = 50 Mean (SD)	Lactobacillus Group n = 50 (Mean (SD)	p - value
Age (month)	16.08 (7.1)	16.32 (6.4)	0.85
Weight (kgs.)			
on admission	7.65 (1.7)	7.75 (1.6)	0.77
at 24hr.	7.68 (1.7)	7.63 (1.6)	0.15
at 48hr.	7.74 (1.7)	7.70 (1.6)	0.91
at 72 hr.	7.70 (1.6)	7.77 (1.5)	0.81

Frequency of stool in 24 hours prior to admission

Minimum	11 (5.5)	12 (5.8)	0.28
Maximum	13 (7.0)	15 (6.5)	0.66

Weight for age
Z score (Ref-NCHS)

- on admission	- 3.04	- 3.01	
- at 72 hrs.	- 3.03	- 2.99	

Axillary temperature

- on admission	- 98.2 (1.1)	98.4 (1.1)	0.64
at 72 hrs.	98.1 (0.6)	98.2 (0.4)	0.67

Placebo group **Lactobacillus group** **p- value**

Table - 2
Characteristic of stool

on admission

Watery	n (%) 37 (74)	n (%) 39 (78)	0.64
Non watery	13 (26)	11 (22)	

At 72 hours

Watery	n (%) 38 (76)	n (%) 30 (60)	0.08
Non watery	12 (24)	20 (40)	

Degree of dehydration

on admission

Some	n (%) 45 (90)	n (%) 46 (92)	0.72
Severe	5 (10)	4 (8)	

At 72 hours

Mild	n (%) 40 (80)	n (%) 44 (88)	0.42
Some	10 (20)	6 (12)	

	Placebo group	**Lactobacillus**	**P- value**
Treatment prior to admission			
ORT (ORS)	40	40	1.00
Antibiotics	10	10	1.00
Antidiarrhoeal	8	12	0.31
I. V. fluid	6	6	1.00
Others	8	9	1.79

Table - 3 Aetiology of Diarrhoea

Electron Microscopic observation of stool samples (n = 100)

	Placebo n = 50	**Lactobacillus GG** n = 50	**Total**
Rota virus	4/50 (8%)	5/50 (10%)	9%
Adenovirus	3/50 (6%)	7/50 (14%)	10%
Enterovirus (SRFV)	1/50 (2%)	1/50 (2%)	2%
Corona virus	6/50 (12%)	2/50 (4%)	8%
	14/ (28%)	15/ 50 = 30%	29%

Results of bacteriological analysis of stool samples (n = 100)
(Direct microscopic and culture of stool)

	Placebo n = 50	**Lactobacillus GG** n = 50	**Total**
Organism			
Shigella	5/50 (10%)	5/50 (10%	10%
Vibrio cholera	7/50 (14%)	10/50 (20%)	17%
Escherichia coli	5/50 (10%)	6/50 (12%)	11%
Campylobacter Pylori	3/50 (6%)	3/50 (6%)	6%
Klebsiella	5/50 (10%)	4/50 (8%)	9%
Proteus	2/50 (4%)	4/50 (8%)	6%
Pseudomonas	6/50 (12%)	5/50 (10%)	11%
Yersinia	2/50 (4%)	1/50 (2%)	3%
Streptococcus fecalis	1/50 (2%)	0	1%

Ascaris Lumbricoids	1/50 (2%)	2/50 (4%)	3%
Trichuris Trichiuria	1/50 (2%)	1/50 (2%)	2%
Cyclospora	1/50 (2%)	0	1%
Citrobacter	1/50 (2%)	3/50 (6%)	4%
E. histolytica	5/50 (10%)	1/50 (2%)	6%
Hookworm	2/50 (4%)	1/50 (2%)	3%
Hymenolepis Nana	1/50 (2%)	0	1%

Presence of reducing substance in the faeces (n = 100)

Reducing substance (+ ve)	20 (40%)	17 (34%)	37%
(≥ 0.5 mg %)	50	50	

Table 4 : Outcome of patients with watery diarrhoea on admission

Outcome Variables	Placebo group n = 50 Mean (SD)	Lactobacillus group n = 50 Mean (SD)	p- value
1. Duration of diarrhoea (days)	3.5 (1.0)	3.3 (1.3)	0.3117
2. Frequency of stool			
on admission	7.4 (4.7)	8.0 (5.7)	0.6058
at 24 hrs	4.0 (2.4)	3.6 (2.3)	0.5041
at 36 hrs	3.8 (2.3)	3.7 (2.4)	0.8983
at 48 hrs	4.2 (2.8)	3.4 (2.3)	0.1883
at 60 hrs	3.3 (2.2)	2.8 (1.8)	0.2392
at 72 hrs	2.7 (2.3)	2.2 (1.9)	0.3172

	Placebo group Mean (SD) n = 50	Lactobacillus group Mean (SD) n = 50	p- value
3. Outcome with shigella			
(a) Duration of diarrhoea (days)			
Shigell present	3.9 (1.3)	2.6 (0.8)	0.0580
Shigella absent	3.4 (1.1)	3.3 (1.4)	0.6766
(b) Frequency of stool on admission			
Shigella present	4.2 (2.1)	8.0 (3.7)	0.1055
Shigella absent	7.7 (4.7)	8 (6.1)	0.8079
At 72 hours			
Shigella present	3.8 (2.0)	2.6 (1.3)	0.5992
Shigella absent	2.6 (2.2)	2.2 (1.9)	0.5482

(4) Outcome with cholera			
(a) Duration of diarrhoea			
Cholera present	3.9 (0.8)	3.8 (1.9)	0.8730
Cholera absent	3.4 (0.9)	3.1 (0.9)	0.1646
(b) Frequency of stool on admission			
Cholera present	11.8 (5.1)	7.1 (3.9)	0.0540
Cholera absent	6.8 (4.3)	8.3 (6.2)	0.2253
At 72 hours			
Cholera present	3.5 (1.5)	2.5 (1.0)	0.5414
Cholera absent	2.6 (2.1)	2.2 (2.0)	0.5919
(5) Outcome with bacteria			
(a) Duration of diarrhoea			
Bacteria present	3.5 (1.2)	3.2 (1.5)	0.5788
Bacteria absent	3.4 (0.8)	3.2 (1.2)	0.5668
(b) Frequency of stool on admission			
Bacteria present	7.3 (3.7)	8.0 (6.9)	0.6663
Bacteria absent	7.6 (5.9)	8.1 (4.1)	0.7775
At 72 hrs			
Bacteria present	2.4 (2.1)	2.0 (1.0)	0.6346
Bacteria absent	3.1 (2.5)	2.6 (2.2)	0.5970
(6) Outcome with virus			
(a) duration of diarrhoea			
Virus present	3.5 (1.1)	3.1 (1.1)	0.5438
Virus absent	3.5 (1.1)	3.3 (1.4)	0.5395
(b) frequency of stool on admission			
Virus present	8.7 (6.6)	6.4 (3.8)	0.6415
Virus absent	7.1 (4.1)	8.4 (6.1)	0.2711
At 72 hrs			
Virus present	2.1 (2.0)	1.7 (0.9)	0.5619
Virus absent	2.5 (2.3)	2.4 (2.3)	0.5888

(7). Outcome with watery diarrhoea	Mean (SD)	Mean (SD)	
(a) duration of diarrhoea			
Watery diarrhoea	3.6 (1.1)	3.2 (1.4)	0.1074
Non watery	3.1 (0.8)	3.5 (1.1)	0.2910
(b) frequency of stool on admission			
Watery diarrhoea	7.1 (3.7)	8.1 (5.4)	0.5880
Non watery diarrhoea	8.2 (6.0)	7.8 (7.4)	0.8799
at 72 hrs			
Watery diarrhoea	2.2 (1.6)	2.0 (1.3)	0.5179
Non watery diarrhoea	4.0 (3.3)	3.1 (2.1)	0.5510

Table 5 : Feeding pattern of study group

	(1)	(2)	(3)	Total
Placebo	6	35	9	50
LGG	6	28	16	50
Total	12	63	25	100

Exclusive breast feeding - (1)
Breast milk + artificial - (2)
Exclusive artificial feeding - (3)

Outcome with breast feeding / Mixed feeding

	Placebo	LGG	p-value
Duration of diarrhoea (days)			
Exclusive breast feeding	3.3 (.42)	3.3 (1)	0.9045
Mixed feeding	3.6 (1.1)	3.3 (1.4)	0.2776
Frequency of diarrhoea Admission			
Exclusive breast feeding	6.1 (3.8)	6.8 (5.0)	0.7971
Mixed feeding	7.6 (4.8)	8.2 (5.9)	0.645
At 72 hrs			
Exclusive feeding	2 (1.7)	1.8 (0.4)	0.816
Mixed feeding	2.7 (2.3)	2.3 (2)	0.6492

Discussion

The management of acute diarrhoea has seen the introduction of a number of new approaches over the past few decades . It is accepted that ORS has real value in decreasing the morbidity and mortality of gastroenteritis by rehydration but it has no effect on the volume, frequency and duration of diarrhoea (32) . Hence, mothers may perceive that ORS is not an effective treatment, thereby reducing the acceptance . However, research in this field has resulted a new generation of ORS (33) . Polymers of glucose and individual aminoacids have been used . Cereals such as rice flour have been used with success to replace sucrose or glucose in ORS . This new cereal based ORS has been found to reduce stool output, duration of diarrhoea and vomiting significantly (34), but for practical reasons such as difficulty in preparation, is not being widely used . Cereal based ORS as tried in the hospital and in the community requires cooking of the rice flour . Therefore it is not yet suitable for packing in sachets, for which precooked or prehydrolysed cereal product is necessary . Moreover cereal-based ORS are more effective, particularly in case of cholera (35) . Unfortunately, inappropriate treatment such as antibiotics and antidiarrhoeals are still widely used .

Breast feeding should be continued during acute diarrhoea and has been shown to shorten the duration of diarrhoeal episodes and decrease the incidence and mortality of persistent diarrhoea (31, 36) .

Management of acute diarrhoea in most countries includes fluid and electrolyte replacement, and modification of pre-diarrhoeal feeding in order to avoid food induced effects of the diarrhoea . The present approach is to look at the whole child, not only his stool .

In 95% of cases, WHO ORS is usually effective in rehydration period of 4-6 hours . After initial rehydration, early reintroduction of feeds is beneficial . However early nutritional repletion is safe and beneficial in patients with acute diarrhoea . Early feeding using human milk in the acute phase of diarrhoea has been shown to decrease the severity of diarrhoea (37) .

The present study has been carried out with due interest in light of the previous findings from Finland & Thailand that human Lactobacillus strain GG promotes recovery from acute diarrhoea in children (14, 17) .

The antimicrobial properties of Lactobacilli has long been recognized (38) . the colonization of the gut with bifidobacteria and Lactobacilli in the breast fed infant is though to be one of the many advantages of exclusive breast feeding and contributory to its protective effect against diarrhoeal disease. It has been shown that Lactobacilli may also have an immunostimulatory effect (25, 27). There has been a resurgence of interest in the possible

Overall, Lactobacillus GG had no significant effect on reducing either the duration or frequency of diarrhoea of these Nepalese children when compared to placebo .

Invasion of the gut by multiple mixed organism may be a factor for the damage of the gut mucosa and thereby reducing the efficacy of Lactobacillus GG (14) .

Certainly, this study would support the need for further studies in tropical countries, noting that further investigation should include a larger sample size, detailed follow up beyond 72 hours, changes in the stool volume, pettern of relapse, different season of a year as the aetiology may be different .

Conclusion

In this present study, both the groups Lactobacillus GG (n = 50) and placebo (n =50) were similar in their history and severity of diarrhoea at presentation . All of them were successfully managed according to their assigned treatment protocol . Lactobacillus GG was well tolerated in the study group . Mixed pathogens were identified in more than 80% of cases.Vibrio Cholera was the main aetiological factor .

Overall the use of Lactobacillus GG did not produce any significant beneficial effect in terms of duration of diarrhoea, stool frequency or the hospital stay .

It may be concluded that Lactobacillus GG may not be effective in case of watery diarrhoea with mixed aetiological factors . However, a longer period of detailed follow-up say beyond 72 hours, study in different period in a year (aetiological effect) , would be worthwhile in studying the use of Lactobacillus GG in tropical countries .

Recommendations

Based on the present study and literature review the followings comments may be drawn .

1. Rehydration therapy is the cornerstone of treatment of acute diarrhoea . This involves fluid and electrolyte replacement using rehydration fluid with appropriate electrolyte composition to match losses in the stool .

2. Cereal based oral rehydration solution or super "ORS" is more effective in the management of acute diarrhoea than older ORS based on sugars . However, its widespread use remains limited by practical difficulties in preparation .

3. Diarrhoea also results in nutritional loss, which in the case of repeated attacks may cause growth retardation . The nutritional aspects of diarrhoea should be treated simultaneously with rehydration therapy . In order to avoid nutritional injury, feeding should be continued during diarrhoea and extrafeeding should be encouraged after recovery .

4. The Lactobacillus GG may be of use in the management of acute diarrhoea due to rotavirus e.g in countries like Finland .

5. The Lactobacillus GG may not be effective once the mucosa of the gut is disrupted by invasion. Moreover, it may be of no use in case of mixed causative organisms .

6. In Tropical countries, with a different problem of gastroenteritis e.g aetiology, or a different background of patients e.g nutritional status, a longer period of detailed follow up in different seasons would be useful say one month should further studies of Lactobacillus GG in gastroenteritis be undertaken .

7. Lactose intolerance seems common with acute gastroenteritis in summer season in Kanti Children's Hospital, Kathmandu .

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Appendix

Questionnaire

Project Title : **Evaluation of the Lactobacillus GG in the management of Acute Diarrhoea .**

Kanti Children's Hospital

Case No. : ☐

Admission : D ☐☐ M ☐☐ Y ☐☐

Time : _____

Name : _____ Sex 1. M ☐

2. F

Father / Mother Name : _____

Address : _____

Date of Birth : D ☐☐ M ☐☐ Y ☐☐

Age : ☐

Frequency of stool in previous 24 hours ☐

Management prior to admission ☐

1. ORT, (ORS, commercial electrolyte solution)
2. Antibiotic
3. Antidiarrhoeal
4. I. V. Fluid
5. Other, specify

Breast feeding ? ☐

1. Yes
2. No

If no up what month : _____

day	I					II				III			
	0	6	12	18	24	30	36	42	48	54	60	66	72
1. Body weight													
2. Temp (axilla for 2min)													
3. Stool collection in sterile 3. Bottle		x	x	x	x	x	x	x	x	x	x	x	
4. Frequency of stool													
5. Character i. Watery ii. Loose iii. Mucus iv. Mucus bloody v. Explosive vi. Other specify													
6. Vomiting Y / N													
7. Frequency of vomiting													
8. Intake I. V. C. C. Oral													
9. Dehydration 1. No 2. Some 3. Severe													
10. Stool PH		x	x	x	x	x	x	x	x	x	x	x	
11. Stool reducing agent with clini test		x	x	x	x	x	x	x	x	x	x	x	
12. Feeding (specify)													

Timing of last diarrhoea stool, Date : _____ Time : _____

Discharge Time : _____ Date : _____ Time : _____

Drug Administration Record

(Unit in 10 C. C. 0f ORS)

Date						
drug compliance						
	dose i 6hr	ii 18hr	iii 30hr	iv 42hr	v 54hr	vi 66hr
1. Drug ot be given 2. Vomiting of drug 1. Yes ☐ 2. No 3. Amount of vomiting 1. Total ☐ 2. Partial 4. Timing of vomiting _____ hour _____min						

Consent

म यस झाडा पखाला सम्बन्धी उपचार कार्यको सिलसिलामा गरिने विधिमा मेरो पूर्ण मन्जुरीछ ।

सही :

नाम :

बिरामीसंगको नाता :