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Lack of effect of nucleotide-supplemented infant formula on the management of acute diarrhea in infants

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Abstract

Nucleotides have been identified as conditionally essential nutrients. As prevention studies, conducted with nucleotide-supplemented formula, have shown statistically significant decrease in the risk of diarrhea, we tested the hypothesis that the consumption of nucleotide-supplemented formula during an acute diarrhea episode is associated with therapeutic effects in the treatment of infants with acute diarrhea and dehydration. A randomized, double-blind, controlled clinical trial was conducted in which patients were randomly assigned to 1 of 2 treatment groups. The "test" group consumed a nucleotide-supplemented infant formula and the "control" group consumed a nonsupplemented formula. Infants were accommodated in a metabolic unit where body weight, and all intakes and outputs were recorded at 24-hour intervals during hospitalization. Laboratory parameters including blood gases and electrolytes were monitored during hospitalization. Eighty-one male infants ranging in age from older than 1 month and younger than 1 year, with acute non-cholera diarrhea and dehydration were studied. Primary outcomes were stool output and duration of diarrhea and did not differ significantly between the groups, with a stool output of 304.2 (SD 254.0) vs 350.3 (SD 269.1) g/kg and a duration of diarrhea of 83.3 (SD 44.5) vs 88.8 (SD 46.6) for the test and control groups, respectively. Anemia was highly prevalent and breast-feeding practice was not frequent in both groups. The average energy intake and weight gain were similar in the 2 groups. This study demonstrated that nucleotide supplementation of infant formula during episodes of acute diarrhea has no therapeutic advantage compared to conventional infant formula.

Keywords: Acute diarrhea; Children; Nucleotides; Nutritional management; Infant formula

1. Introduction

The current recommendation for the proper management of diarrheal diseases includes oral rehydration therapy, selective use of antimicrobials, and adequate nutritional support during, and immediately after, recovery from the acute phase. More recently, zinc supplementation for 10 to 14 days has been recommended in the new treatment guidelines for diarrhea of the World Health Organization (WHO) and the United Nations Children's Fund [1]. This strategy has shown a dramatic impact on the course of the disease and on infant mortality. However, there was almost no effect on morbidity.

The maintenance of the usual diet during acute episodes of diarrhea has been extensively discussed [2]. Maintenance of the usual diet when compared to food withdrawal does not increase stool losses but promotes a better nutritional balance [3]. The recommendations of the WHO for continued feeding during diarrhea emphasize that foods should be culturally acceptable, readily available, have a high content

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of energy, and provide adequate amounts of essential micronutrients [4].

Despite the known association between diarrheal diseases and transient lactose intolerance [5], data from a meta-analysis have shown that most patients with acute diarrhea, especially mild and moderate cases, can receive a regular undiluted cow's milk-based diet [6]. Although the lactose content of maternal milk is higher than that of cow's milk and very similar to that of milk-based infant formula, stool losses associated with breast-feeding are significantly lower; thus, breast-feeding should be maintained and even promoted [7]. Several lines of evidence indicate the maintenance of breast-feeding is an important protective measure to reduce morbidity and mortality. Many effects of breast-feeding are associated with immune factors, but it is possible that protection is also related to nonspecific factors such as nucleotides.

Recently, the addition of nucleotides to infant formula has been suggested as it has beneficial effects on the growth and maturation of the gastrointestinal tract as well as on the immune response [8-10]. Studies have demonstrated that formula supplemented with nucleotides appears to reduce the incidence of diarrheal diseases in healthy infants [11-15].

Nucleotide synthesis occurs according to metabolic needs; however, the intestinal mucosa has limited capacity for synthesis. In cases of mucosal injury, nucleotides become essential for the organism [11,16]. This mechanism might explain the decrease in the incidence of diarrhea observed in previous studies.

As prevention studies, conducted with nucleotide-supplemented formula, have shown a statistically significant decrease in the risk of diarrhea, the objective of the present study was to test the hypothesis that the consumption of nucleotide-supplemented formula during an acute diarrhea episode is associated with therapeutic effects in the treatment of infants with acute diarrhea and dehydration when compared to the consumption of a conventional infant formula.

2. Methods and materials

This double-blind, randomized clinical trial was carried out at the Fima Lifshitz Research Center, University Hospital Complex Professor Edgard Santos, Federal University of Bahia, Brazil. The ethics review committee of the institution accorded approval to the study.

Eighty-one male infants aged between 1 to 24 months with a history of acute watery diarrhea lasting less than 3 days and with overt clinical signs of dehydration were included in the study. The nature of the study was clearly explained to the parents or legal guardians, and their written consent was obtained before the study. Criteria for exclusion from the study were severe concurrent diseases such as pneumonia, meningitis or sepsis, signs of intestinal obstruction or ileum, clinical signs of severe malnutrition, large amounts of blood or mucus in the stools, exclusive breast-feeding, and/or refusal of informed consent.

A sample size of 40 patients per group was estimated to be sufficient to detect a difference of 30% in diarrhea duration, assuming a power of 80% and a significance of 5%.

The children were randomly assigned to 1 of 2 treatment groups. One group received an infant formula supplemented with 72 mg/L nucleotides based on the total potentially available nucleotide content of human milk. The other group received a conventional infant formula. The patients were accommodated in a metabolic unit, and all their intakes and outputs were measured and recorded at 24-hour intervals until the diarrhea ceased or they fulfilled the definition of persistent diarrhea (14 or more days of diarrhea duration). Stool losses were determined, and urine was separated from stool using urine collection bags. Vomit was weighed using preweighed gauze pads. Body weight was recorded on admission, after rehydration at 6 hours, and thereafter at 24-hour intervals until discharge. Laboratory parameters including blood gases and electrolytes were monitored during hospitalization. Both formulas were stored in identical cans, and personnel not directly involved in the protocol prepared the feeds to preserve the blind nature of the study. A patient's individual energy requirements were calculated according to the 1985 Food and Agriculture Organization of the United Nations/WHO/United Nations Association criteria [17]. To exclude confounding variables, all children received a diet based on formula and breast-feeding only and no semisolid food was given. Patients whose oral intake did not reach 418.4 kJ/kg per day after 72 hours of dietary treatment and who lost weight received a nasogastric tube to facilitate appropriate formula intake. Children were kept on the study from randomization until diarrhea ceased or until they fulfilled the criteria of persistent diarrhea ($\geq$14 days of diarrhea duration).

2.1. Statistical analyses

The distribution of important outcome variables such as stool output (g/kg), formula intake (g/kg), and duration of diarrhea (hours) was evaluated. The 2-tailed Student t test was used for comparison between independent groups under an assumption of normality. The median of continuous variables with skewed distribution was compared using the Mann-Whitney test. Qualitative variables were compared using the χ^2 test [18]. Data are presented as means and SDs. Kaplan-Meyer survival analyses were performed to compare the duration of diarrhea for different degrees of severity [19,20].

3. Results

A total of 89 patients were enrolled in the study. Seven patients were excluded because of the lack of any diarrheal stool after admission, and 1 patient was excluded at the

request of the parents. Thus, 81 patients were included in the analysis.

The clinical and laboratory characteristics of the patients on admission were similar in the 2 groups (Table 1). Anemia was highly prevalent in both groups. Breast-feeding practice was not frequent. However, mothers did not withhold breast-feeding during the diarrheal episodes. Only 7.4% of the mothers maintained the usual diet during diarrheal episodes, with no significant difference between the groups. The prevalence of malnutrition according to the weight-for-height score was very high (12.3%), and the prevalence of chronic malnutrition according to the height-for-age score was low (2%), when compared to Brazilian data showing prevalence of 5% and 15.4%, respectively [21]. The average energy intake was similar in the 2 groups. The proportion of patients who required a nasogastric tube to ensure adequate energy intake (35% vs 32%) was also similar in the 2 groups.

Our data showed a similar weight gain in the 2 groups, indicating a very important nutritional rehabilitation rate in both groups.

No difference between groups was observed with regard to the outcome variables examined in this study (Table 2). When stratified according to stool output rates, no difference could be discerned in the duration of the more severe episodes (Fig. 1).

4. Discussion

Despite continuous effort in training parents and health workers in proper management of diarrhea cases, our data showed that inappropriate practices were still very common in the 2 groups at the household level. Fortunately, oral rehydration solution and other home fluids were offered immediately after the onset of diarrhea in 80% of cases, a fact that may explain the admission of only 1 patient with severe dehydration.

We realize that the exclusive formula-based diet proposed was not appropriate for all the patients studied, in relation to their age. Nevertheless, the short period of observation and the very close and individualized follow-up of each patient ensured that the adequate nutritional complement was administered.

Although the trophic effect of nucleotides on the intestinal mucosa has been recognized, our data showed no significant impact of nucleotide supplementation of infant formula on the clinical course of diarrhea. Assuming that the average duration of the diarrheal episodes during hospitalization was 80 hours, one can infer that for an event promoting any trophic effect on the intestinal mucosa to occur—which takes an average of 2 to 3 days for completion of the epithelial turnover—the impact of the intervention should have been faster than what was observed in the course of the study.

It is also very important to understand that any proposal to optimize the clinical management of diarrhea should trigger

Table 1
Baseline characteristics of patients in the group receiving nucleotide-supplemented infant formula (study group) and the group receiving conventional infant formula (control group)

Baseline characteristics at enrollment	Study group (n = 40)	Control group (n = 41)	*P*
Mean age (mo)	10.4 ± 6.2	10.0 ± 5.5	NS
Mean length of diarrhea at enrollment (h)	51.3 ± 25.5	53.7 ± 21.9	NS
Mean length of vomiting at enrollment (h)	49.5 ± 37.0	46.0 ± 28.5	NS
Percentage of patients with fever	77.5 ± (31)	75.6 ± (31)	NS
Mean length of fever at enrollment (h)	34.1 ± 31.4	43.0 ±38.4	NS
Percentage of patients with bloody stools	7.5 ± (3)	4.8 ± (2)	NS
Percentage of patients using antibiotics	25.0 ± (10)	29.2 ± (12)	NS
Percentage of patients with dehydration	95.0 ± (38)	97.6 ± (40)	NS
Mild/moderate	95.0 ± (38)	95.2 ± (39)	NS
Severe	0.0 ± (0)	2.4 ± (1)	NS
Serum sodium (mEq/L)	141.0 ± 5.4	141.0 ± 4.4	NS
Serum potassium (mEq/L)	4.5 ± 0.7	4.4 ± 0.7	NS
Hematocrit (%)	30.4 ± 2.4	30.5 ± 3.2	NS
Hemoglobin (g/dL)	10.1 ± 0.9	10.2 ± 1.1	NS

Data are means ± SD or percentage. Two-tailed Student *t* test and χ^2 test were performed for quantitative and qualitative variables, respectively. NS indicates not significant.

Table 2
Clinical features and outcome variables of the patients during hospitalization

	Study group (n = 40)	Control group (n = 41)	*P*
Total stool output (g)	2469.6 ± 1966.3	2637.3 ± 1639.8	0.67[a]
Total stool output (g/kg)	304.2 ± 254.0	350.3 ± 269.1	0.43[a]
Total stool output rate (g/kg per hour)	3.4 ± 1.9	3.7 ± 1.4	0.47[a]
Diarrhea duration (h)	83.3 ± 44.5	88.8 ± 46.6	0.58[a]
Hospitalization period (h)	118.4 ± 50.2	120.6 ± 45.2	0.83[a]

Data are means ± SD.
[a] Two-tailed Student *t* test was performed ($P < .05$).

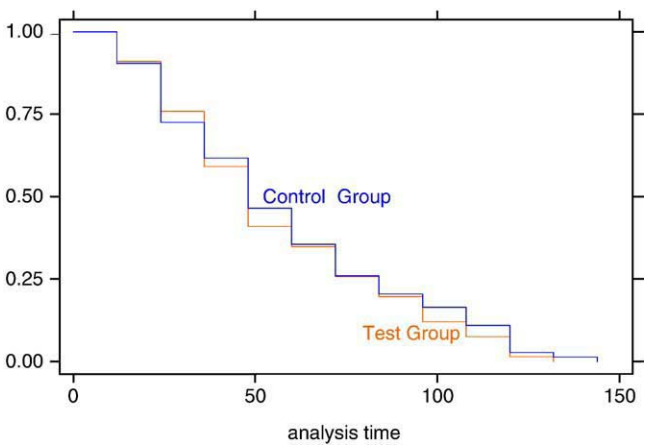

Fig. 1. Kaplan-Meier survival analysis for the duration of diarrhea with large stool output (>5 mL/kg per hour).

a rapid clinical response to reduce the duration of diarrhea, stool losses, and, consequently, the duration of hospitalization, patients' expenses, and infant mortality.

In our study, we have tested only one concentration of nucleotides (72 mg/dL) and, as a recent meta-analysis on this issue, speculate that the total potentially available nucleotides in human milk may be higher than these levels, having tested only one concentration of nucleotides might have been a limitation for finding positive results in our study [22]. In future studies, more levels should be tested.

It is very important to take into account that, in our study, both groups consumed infant formula and there was no breast-feeding observation group. Although the new generations of infant formula are designed to mimic the health advantages of human milk, the differentiated patterns of gut microbiota between breast-fed and formula-fed infants may interfere with the biological effect of nucleotides on the intestinal mucosa [22,23].

Further studies using a longer period of exposure of the intestinal mucosa to nucleotide supplementation and using different concentrations are necessary to allow the occurrence of the potential trophic effect of supplementation. In view of the beneficial effects reported in the literature, it is possible that young infants on nucleotide-supplemented formula as part of their usual diet might present less severe or shorter diarrheal episodes than infants using conventional formula. The same conclusions may apply to patients with persistent diarrhea and malnourished patients.

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