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Effect of bovine lactoferrin from iron-fortified formulas on diarrhea and respiratory tract infections of weaned infants in a randomized controlled trial

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ABSTRACT

Objective: The aim of this study was to evaluate the effect of supplementation with bovine lactoferrin (bLf) from iron-fortified formulas on diarrhea and respiratory tract infections (RTIs) in weaned infants.

Methods: In this prospective, multicenter, controlled intervention study, 260 infants ages 4 to 6 mo who previously were exclusively breastfed but weaned were randomized into two groups: a lactoferrin-fortified formula milk group (fortified group, FG, containing lactoferrin 38 mg/100 g milk) and a no lactoferrin-fortified milk (control group, CG); breastfed infants were enrolled and served as a reference group (breastfed group, BG). The intervention duration was 3 mo. The morbidity of diarrhea and RTIs were collected during supplementation.

Results: The results of the study demonstrated evidence of a lower incidence rate of respiratory-related illnesses and fewer symptoms of running nose, cough, and wheezing for infants in the FG and BG groups compared with those in the CG ($P < 0.05$). Despite the undistinguished incidence rate of vomiting, nausea, and colic, the occurrences of diarrhea-related illnesses were significantly lower for children in the FG and BG than for those in CG ($P < 0.05$).

Conclusion: The beneficial effects on infectious morbidity over 3 mo highlighted the potential of bLF supplementation for previously weaned infants; these findings may be applicable to other infants living in similar socioeconomic districts.

Introduction

The Child Health Epidemiology Reference Group of the World Health Organization (WHO) and UNICEF estimated that of 7.6 million deaths in children younger than age 5 y in 2010, 64% (4.879 million) were attributable to infection-related causes [1]. Prevention against infectious disease is very important for the health and development of tropical populations. In addition to causing mortality, infectious disease has serious long-term effects as multiple episodes affect children's growth, nutritional status, and cognition.

Breastfeeding is a cost-effective intervention for protecting children against infectious disease and all causes of mortality [2]. Breastfeeding protects infants by serving as a source of nutrition uncontaminated by environmental pathogens.

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Multiple anti-infective, anti-inflammatory, and immune regulatory factors transmitted through milk, including secretary antibodies, glycans, lactoferrin (LF), leukocytes, cytokines, and other components produced by mother's immune system also help to strengthen the infants' immune systems [3,4].

LF, the second most abundant protein in human milk, acts as an antimicrobial, anti-inflammatory, and immunomodulatory agent [5–7]. It protects against gram-negative enteropathogens by sequestering iron essential for bacterial growth, binding to the lipid A portion of lipopolysaccharide on the cell surface, and disrupting the bacterial cell membrane [8,9]. In vitro studies show that LF decreases virulence of enteropathogens by decreasing their ability to adhere to or invade mammalian cells, and by binding to, or degrading, specific virulence proteins [10,11]. Human lactoferrin (hLF) and bovine lactoferrin (bLF), despite minor structural and biochemical differences, have similar bioactivity, as assessed through in vitro and animal models [12]. Moreover, bLF has been shown to be safe in infants [13,14].

Current studies of iron-fortified formula lack an investigation into the protective effects of bLF on the morbidity of diarrheal and respiratory tract infections (RTIs) in weaned infants. We carried out a randomized, placebo-controlled, blinded trial in a peri-urban community located in the western periphery of Chengdu City to evaluate the effect of bLF from iron-fortified formulas on diarrheal and RTI diseases. In this study, we addressed the hypothesis that weaned infants supplemented with formulas fortified with bLF will have reduced morbidity of diarrhea and RTIs compared with infants who received formulas without bLF.

Materials and methods

Participants and ethical approval

This randomized, controlled, blinded intervention study was performed in the Qing Baijing, Jinniu, Dayi, Chenghua, Meishan, and Xindu districts of Chengdu City, Sichuan province in western China from March 2012 to March 2013. Approximately 260 term infants who were previously exclusively breastfed, then required formula feeding at 4 to 6 mo, were randomly recruited from six Maternal and Child Departments of hospitals in the six described regions. In parallel, a group of 130 healthy, exclusively breastfed term infants were also enrolled and served as a reference group (BG). In all, 390 infants met the inclusion criteria (130 for each group). Thirty-one infants were excluded due to parents' refusal to participate.

Eligibility and exclusion criteria

The eligibility criteria for participation were as follows:

1. Apparent good health without common obstetric risk factors;
2. Hemoglobin (Hb) concentration >60 g/L;
3. Serum C-reaction protein (CRP) < 10 mg/L;
4. Parent or guardian approval for participating in all aspects of the study; and
5. Parent or guardian agreement to avoid additional use of infant formulas, and foods fortified with iron during the investigation (for infants in BG, agreement with exclusive breastfeeding without formulas and supplementary food).

Exclusion criteria included a history of severe, persistent, or chronic diarrhea; severe malnutrition; serious infections requiring hospitalization in the month prior; serious chronic illness; or a personal or family history of allergy to cow's milk or infant formula, eczema, allergic rhinitis, or asthma.

Primary and secondary outcomes

The primary outcome of the present study was the morbidity of diarrhea and RTIs during the intervention. The secondary outcome was the effect of intervention on the duration of respiratory- and diarrhea-related illnesses.

Sample size

The present study was part of our research about the influence of bLF on anemia and growth and development of infant from iron-fortified formulas [15]. According to original protocol, the initial primary objective for the research was to measure the change in serum Hb before and after the intervention. A sample size of about 60 infants per group was sufficient to allow the detection of a 10 g/L Hb SD of difference after fortification with 95% power and $\alpha = 0.05$ for a two-sided t test. The initial recruitment size was about 130 children per group, however, this declined throughout the testing as a number of children were withdrawn from the study. We referred some similar studies that because a 5% absolute increase in the longitudinal prevalence of diarrhea is associated with an increased risk for mortality of 17% [16] and we thus considered a 5% difference in the longitudinal prevalence of diarrhea among groups to be clinically meaningful. Therefore, we estimated that 30 infants per group would ensure a power of 95% ($\alpha = 0.05$) to detect between-group differences for the longitudinal prevalence of diarrhea (log-transformed). That is to say, initial original sample size (130 for each group) can allowed sufficient power (>95%) to evaluate the incidence of infectious illness as a primary outcome in the present study.

We conducted a census in each regional hospital to determine which households included a weaned or exclusively breastfed 4- to 6-mo-old infant. Then, field health workers conducted a food-intake survey to determine which infants were weaned. Eligible families were visited by a fieldworker who explained the protocol, answered questions, and obtained written informed consent from parents/guardians. The enrollment and research plan were reviewed and approved by the institutional ethics committee of the Chengdu Women's and Children's Central Hospital of Chongqing Medical University in Sichuan province, China. The present study complied with the code of ethics of the World Medical Association (Declaration of Helsinki).

Intervention

Two different fortification styles were performed in the present study and eligible weaned infants were randomly assigned to one of the two intervention groups. Infants in the fortified group were given a commercially available bLF-fortified formula (Beingmate Baby & Child Food Co., Ltd, Hangzhou, China), which contained a bLF concentration of 38 mg/100 g. Infants in the control group were given an LF-free formula (Beingmate Baby & Child Food Co., Ltd, Hangzhou, China). Both formulas contained the same composition of nutrients except for LF. The iron content was also the same at 4 mg/100 g.

Randomization and allocation concealment

Immediately after recruitment, infants were assigned a study number that had been previously randomly assigned to the FG or CG with fixed, equal allocation to each group prepared by a research secretary at the Chengdu Women's and Children's Central Hospital, who was not connected to the study. The RAND function of Excel (Microsoft, Redmond, WA, USA) was used to generate randomly permutated codes with concealment to ensure that the allocation was not made before the parents of the infants had given their consent and joined the study.

Blinding

The physicians, nurses, field health workers, parents, and laboratory personnel were blinded to the treatment assignment of each infant throughout the study period. The data manager, statistician, and all investigators remained blinded to group assignments until the end of data analysis. The total duration of fortification was 3 mo. Infants in BG continued to exclusively breastfeed without any formula, or supplementary food fortified with iron for ≥3 mo.

Anthropometric measurements

Anthropometric examinations for each infant were conducted by the same trained nurse (a total of six) at baseline and follow-up (3 mo) using standardized techniques to eliminate intraexaminer error. Duplicate measurements were performed for all infants. The interexaminer coefficient of variation of weight, length, and head circumference (HC) for each examiner in FG, CG, and BG was <5%. Weight was recorded using a weighing scale (100 Med, Beijing, China) to the nearest 100 g with infants in minimal clothing and bare feet. Similarly, length was measured in the standard supine position by a supine scale (Haode, Guangzhou, China) to the nearest 0.1 cm. By using reference data from, the Z-scores were calculated for height for age, weight for height, and weight for age. All indices were computed using Anthro for the personal computer, as recommended by the WHO (http://www.who.int/childgrowth/software/en/).