Indian J Gastroenterol (November–December 2011) 30(6):270–276
DOI 10.1007/s12664-011-0146-0

ORIGINAL ARTICLE

Effect of bovine colostrum-based food supplement in the treatment of HIV-associated diarrhea in Northern Uganda: a randomized controlled trial

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Received: 23 November 2010 / Accepted: 10 November 2011 / Published online: 13 December 2011

Abstract
Aim HIV-associated diarrhea is common in HIV/AIDS patients in developing countries. An earlier uncontrolled study showed that a nutritional product made from bovine colostrum (ColoPlus®) alleviates HIV-associated diarrhea. We performed a randomized single-blind controlled trial of addition of colostrum-based supplement (ColoPlus®) to standard anti-diarrhea treatment in HIV/AIDS patients with diarrhea.
Methods Eighty-seven adult patients with HIV-associated diarrhea were recruited at Gulu Hospital and four community clinics in Northern Uganda. Forty-five patients were randomized to receive 50 g of colostrum-based supplement twice a day for 4 weeks in addition to standard anti-diarrhea treatment, and 42 patients received standard anti-diarrhea treatment alone. Patients were followed up for 9 weeks. Daily stool frequency was recorded, and body weight and body mass index were evaluated at weeks 1, 4 and 9. Baseline CD4 + count was measured at baseline and at week 9.
Results Mean daily stool frequency decreased by 79% from 7.5 to 1.3 motions over the study period in patients on colostrum-based supplement, compared to a 58% reduction in controls ($p<0.001$). Self-reported fatigue was reduced by 85% in patients on colostrum-based supplement by week 9 compared to 43% reduction amongst controls ($p<0.001$). Patients on colostrum-based supplement had 11% increase ($p<0,001$) in mean body weight and body mass index by week 9, but no changes were observed in control subjects. Mean CD4+ count increased by 14% for patients on colostrum-based supplement, in contrast to 12% decrease in controls ($p<0.001$).
Conclusions This study shows that addition of colostrum-based supplement to standard therapy is effective in treatment of HIV-associated diarrhea.

Keywords CD4+ · Colostrum · Colostrum-supplementation · Diarrhea · HIV · HIV-associated diarrhea

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Introduction

Uganda is located in East Africa and has a population of about 30 million, of which nearly 980,000 are people living with human immunodeficiency virus (HIV). Gulu in northern Uganda is one of the districts hardest hit by acquired immunodeficiency syndrome (AIDS). Data from HIV sentinel surveillance site at Lacor Hospital reveal that Gulu district has HIV prevalence rate of 11.9% [1].

The Uganda Ministry of Health Policy on HIV/AIDS care ranks nutritional intervention as a key component of the comprehensive care package for HIV/AIDS patients [2].

HIV-infected patients are commonly faced with malnutrition and chronic diarrhea, requiring prolonged or recurrent antibiotic treatment. Studies have shown that in 30% of patients with HIV-associated diarrhea, enteric pathogens cannot be identified; and of the remaining 70%, only about half of the identifiable etiological agents were treatable with antibiotics [3]. This means that only about 35% of patients with HIV-associated diarrhea improve on regular treatment including antibiotics therapy.

The above problems faced by HIV-infected patients with chronic diarrhea could be addressed by the use of bovine colostrum products. Colostrum contains high titers of antibodies or immunoglobulin active against a wide range of bacterial, viral and protozoa pathogens as well as against various bacterial toxins [3–8].

ColoPlus® is bovine colostrum-based food product in porridge form, is administered orally, with no known side effects reported so far. An earlier open-labeled observational study from Nigeria indicated clinical improvement of patients with HIV-associated diarrhea on regular supplementation with ColoPlus® [9].

We evaluated the effects of a colostrum-based supplement (ColoPlus®), using a randomized single-blind trial, on diarrheal frequency, immunological response and nutritional status of patients with HIV-associated diarrhea.

Methods

Study setting

The study was done between October 2007 and June 2008 in Gulu Regional Referral Hospital HIV/AIDS Clinic and four community outreach sites (mobile clinics) located in four internally displaced persons' (IDP) camps in Gulu and Amuru districts of northern Uganda. Laboratory tests were performed at the Joint Clinical Research Center (JCRC) specialized laboratory in Gulu. Samples collected from patients at the community outreach sites were transported under optimal conditions to the JCRC laboratory within 4 h of collection.

Study patients

Study patients were recruited, examined and followed up at both the hospital HIV/AIDS clinic and the community outreach sites. The inclusion criteria were HIV-positive patients aged 18 years or more; diarrhea with stool frequency of four or more motions per 24 h for more than 7 days; patients not receiving anti-retroviral (ARV) therapy; and those who gave their informed consent. Patients with stool frequency of less than 4 motions per 24 h or those with diarrhea lasting 7 days or less; those who had used commercially produced nutritional supplements (immune boosters) during the previous 48 h; those with known allergy or intolerance to milk and milk-products, and pregnant women were excluded

Study design

The study was an open-labeled, randomized, clinical trial. One group of patients received 50 g of colostrum-based supplement twice a day for 28 days, in addition to the "regular care" currently offered to patients with diarrhea in Uganda. The second group, serving as the control group, received only the "regular care" for HIV patients with diarrhea until the diarrhea subsided. Randomization was done using a table of random numbers, and a unique number was allocated to each study subject. These random numbers were matched to the study identification numbers and were used to allocate either treatment protocol. "Regular care" of HIV-associated diarrhea currently involves fluid and electrolyte replacement, use of anti-diarrheal drugs and antibiotics in appropriate doses as well as good nutrition using locally available food items. Patients were followed up at weekly intervals in the clinics for a period of 9 weeks.

Sampling procedure

It was assumed that the proportion of patients with HIV-associated chronic diarrhea whose diarrhea would subside after regular treatment, including antibiotics was 35% [3]. It was further assumed that the proportion of patients with HIV-associated diarrhea whose diarrhea would subside after receiving colostrum-based supplement was 72% [5, 9]. Assuming 20% loss to follow up of the calculated sample size (6.4≈7 patients), the minimum sample size for each arm was 39≈40. For both arms, a minimum of number of study patients was therefore 80.

Patients who met the selection criteria were randomly assigned to either of the treatment groups. The purpose of the study was explained to them by a study nurse and informed consent was taken prior to enrolment. A total of 45 patients were recruited on the colostrum-based supplement (intervention) arm and 42 were recruited on the control arm (Fig. 1).

At the time of enrolment, baseline socio-demographic data and clinical information were recorded on a pre-coded, pre-tested standardized questionnaire. A clinic staff administered the questionnaire, designed in English and translated into the local language (Luo), to obtain and record the socio-demographic information, and baseline symptoms and examination findings. Other important baseline parameters documented included self-reported frequency of stool per 24 h, and self-estimated fatigue level, body weight,

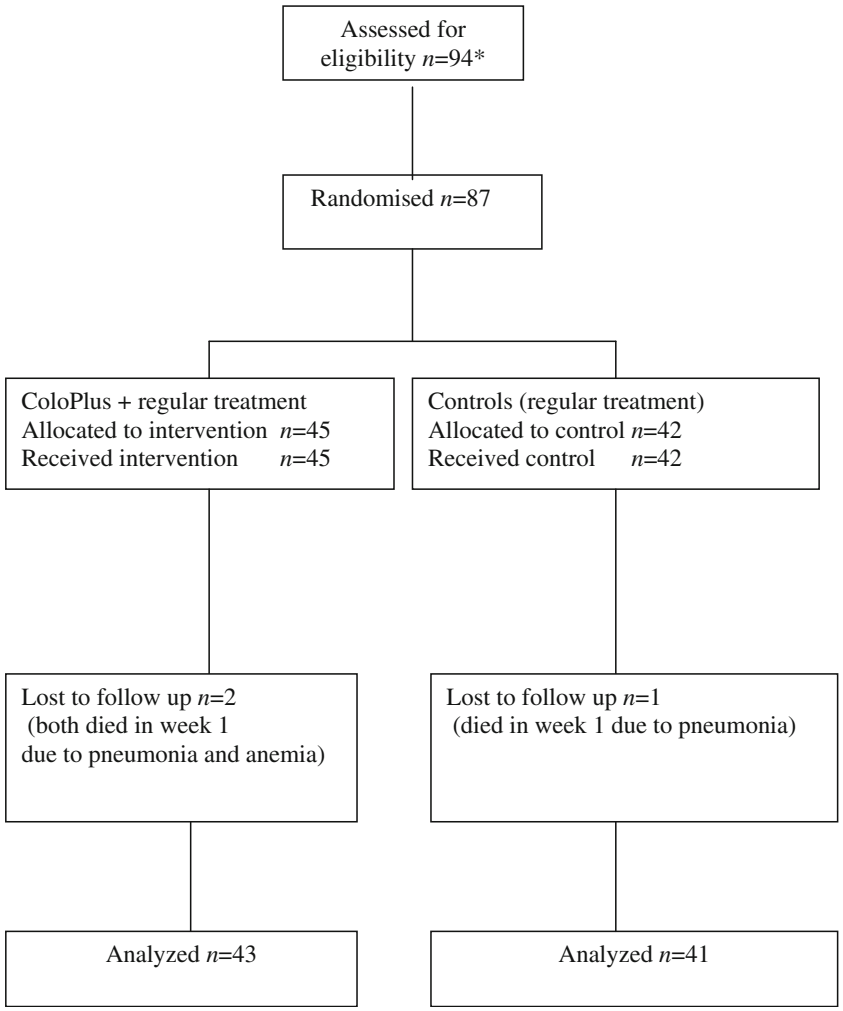

Fig. 1 Patients' flow chart. * (7 excluded, 3 had very low CD4+ counts and were started on ARVs within 1 week, 1 used antibiotics in previous 48 h, 3 declined to consent)

body mass index, stool microscopy results, hemoglobin level, serum albumin, and CD4+ counts.

During weeks 1 to 4, patients in the intervention arm received 50 g of colostrum-based supplement twice daily. They also received regular care until the diarrhea ceased. Compliance to colostrum-based supplement intake was checked at weekly intervals and was over 95%. Patients in the control arm received only the regular care until diarrhea subsided.

The main outcome measures, which included stool frequency per 24 h, fatigue level, body weight, body mass index, stool microscopy, hemoglobin and serum albumin, were assessed at weeks 4 and 9 in all patients. CD4+ count was repeated at week 9. Measurements at 9 weeks were used to examine whether colostrum-based supplement had a protracted effect on the outcome measures 5 weeks after stopping treatment.

Stool frequency was recorded by the patient on a dairy card on a 24 hourly basis throughout the study period. The records for each patient were regularly verified for completeness by the clinic staff during the weekly follow-up visits. For those who could not read and write, the study nurse filled in the dairy card using patient recall. Fatigue level was estimated by the patient using a visual analogue scale (VAS) consisting of a line drawn with equal interval scale from 0 to 10, where 0 represented no fatigue and 10 represents the worst fatigue level possible. Body weight and height were measured by the clinic nurse, and the body mass index (BMI) was calculated from these values. Stool microscopy, serum albumin and hemoglobin measurements were done at the JCRC laboratory using standard procedures. CD4+ count was determined using flowcytometry.

Statistical analysis

Data were entered in EPI Info, exported to and analyzed using the Statistical Package for Social Sciences computer software (SPSS version 10.0). Categorical outcomes were analyzed using chi-squared test, and continuous variables were analyzed using independent sample t-test and analysis of variance. Multiple logistic regressions method was used for multivariate analysis.

Ethical considerations

ColoPlus usage was accepted by the Ethical Committee of Lund University, Sweden in September 2002 (LU-472-02) which approved of the use of ColoPlus on adult patients with HIV-associated diarrhea. Ethical approval for the Uganda study was obtained from Gulu University Faculty of Medicine Institutional Research Committee (IRC) and the Uganda National Council for Science and Technology (UNCST–HS 317).

ColoPlus is patented by ColoPlus AB, Malmö, Sweden and is manufactured in accordance with the Swedish National Board of Food Administration. ColoPlus is in dry-powder form with particles 0.3–7.0 mm in diameter. The particle size allows slower intestinal transit than that for milk. The active part of the product is collected and processed from bovine colostrum in such a way that its antimicrobial properties are preserved. The active part then is added to a vehicle of dry rice flakes, and banana flakes and sugar are added to give a pleasant taste. The particles swell after mixing with water, and the starch-containing vehicle has a protective role for the active substances in the bowel and contributes to increasing the transition time through the gastrointestinal tract.

ColoPlus is packed in portions of 50 g to be taken twice a day, with the first and last meal of the day, giving an interval of about 12 h. Each portion is mixed by the patient with 120 mL of boiled lukewarm water (max 60°C) to porridge-like consistency just before ingestion. Each portion of the product contains 3–4 g of immunoglobulin, the main part of which is constituted by IgG_1. Growth factors, such as IGF-1, TGF-β2, are positively correlated with immunoglobulin content of bovine colostrum [10].

The composition of ColoPlus product is as follows: 32% colostrum powder; 30% rice flakes; 14% banana flakes; 20% maltodextrin; and 4% sugar. Energy value of one sachet of ColoPlus, which equals 50 g, is 825 kJ, which is equivalent to 175 kcal.

Results

Demographic data

The mean age of all study participants was 36.7 years. Participants who received colostrum-based supplement were younger than the controls; 34.5 years vs. 39.1 years (p=0.018). Most of the study participants (n=60 [69%]) were females. The sex distribution, and socioeconomic characteristics measured by education level and occupation, were similar for the two groups. Twenty percent of the participants did not have any formal education (Table 1).

Medical history

The mean 24 h frequency of stool at time of enrolment into the study was 7.2 motions per day, while the average duration of diarrhea was 16.6 days. Patients on the two treatment groups were similar in these baseline parameters. Overall, 36.8% of the patients had fever at baseline. The mean duration of fever at enrolment was similar in both treatment groups, 8.7 days and 7.2 days for colostrum-based supplement and control groups, respectively. Seven patients had history of other chronic illnesses in the previous 1 month: 5 patients were treated for tuberculosis and 2 for peptic ulcer disease. The majority of the patients (93%) were on septrin prophylaxis, with mean duration of septrin use being 13.8 months. Seventeen (20%) patients were using other antibiotics at the time of enrolment; antibiotics used by patients in the previous 1 month included: amoxicillin, ciprofloxacin, ampicillin, doxycycline, chloramphenicol and metronidazole (Table 1). Patients on colostrum-based supplement did not differ from the controls in septrin and other antibiotics use. None of the patients was on any nutritional supplements at enrolment.

Laboratory findings

Patients on both treatment groups did not differ in their mean baseline hematocrit, hemoglobin, and white blood cell counts. However, patients on colostrum-based supplement had relatively lower mean CD4+ counts and mean serum albumin compared to the control group.

Seven (8%) patients had identifiable micro-organisms on stool microscopy; *Trichomonas hominis* was seen in three patients, *Giardia lamblia* in two patients, and *Entamoeba histolytica* and *Hymelnolepsis nana* in one patient each.

Clinical parameters

The mean (SD) number of stool motions per day decreased from 7.5 (2.9) at enrolment to 1.6 (0.7) at week 4 in the colostrum-based supplement group, and from 6.9 (3.2) to 2.9 (1.8) in the control group (p<0.001). The protracted effect was seen after 5 weeks of stopping colostrum-based supplement. At 9 weeks, mean number of stool motions was 1.3 (1.6) in the colostrum-based supplement group, compared to 2.7 (SD 3.5) in the control group (p=0.041) (Table 2).

Diarrhea, defined as stool frequency of more than 3 motions per day, on average ceased by day 7 for patients receiving colostrum-based supplement and stool frequency was normal at week 9, 5 weeks after stopping colostrum-based supplement. The control subjects

Table 1 Baseline demographic and laboratory characteristics of study participants by study group, n=87

Characteristics		Colostrum-based supplement (n=45)	Control group (n=42)	Total	p-value
Age (y) (mean [SD])		34.5 (8.1)	39.1 (9.7)	36.7 (9.1)	
Sex (n [%])	Male	17 (37.8)	10 (23.8)	27 (31)	
	Female	28 (62.2)	32 (76.2)	60 (69)	
Education (n [%])	None	9 (20.0)	8 (19.0)	17 (19.5)	
	Primary	28 (62.2)	33 (78.6)	61 (70.1)	
	Secondary	8 (17.8)	1 (2.4)	9 (10.3)	
Occupation (n [%])	Not employed	30 (66.7)	15 (33.3)	45 (52)	
	Self employed	27 (64.3)	15 (35.7)	42 (48)	
Septrin prophylaxis (n [%])		40 (88.9)	41 (97.6)	81 (93)	
Current antibiotics use (n [%])		8 (17.8)	9 (21.4)	17 (20)	
Laboratory parameters (mean [SD])					
	Hematocrit, %	34.4 (6.5)	36.5 (6.3)	35.4 (6.5)	0.127
	Hemoglobin, g/dL	11.7 (2.2)	12.2 (2.1)	12.0 (2.2)	0.303
	Total WBC (cells×10^9 /L)	4.2 (1.5)	4.7 (2.3)	4.4 (1.9)	0.247
	CD4+ count (cells/μL)	379 (272)	492 (281)	416 (287)	0.044
	Serum albumin (g/dL)	3.6 (0.7)	3.9 (1.0)	3.7 (0.8)	0.040

achieved normalization of stool frequency at mean 21 days.

Patients who received colostrum-based supplement reported greater improvement in their life and feeling of wellbeing, with less fatigue as compared to the control group (Table 3). There was 11.4% increase in mean weight and mean BMI at week 9 among patients on colostrum-based supplement (p<0.001) but there was no change in weight in the control group (p=0.251). The serum albumin and hemoglobin was similar in both treatment groups at weeks 4 and 9.

CD4+ counts

The mean CD4+ count increased by 14% at week 9 in the colostrum-based supplement group but was reduced by 12% over the same follow up period among the controls (p<0.001) (Table 4).

Discussion

Previous studies that examined the effects of bovine colostrum preparations, including one study with ColoPlus, in the treatment of patients with HIV/AIDS-associated diarrhea, have all been observational studies [3, 5, 6, 9]. This is the first randomized single-blinded controlled clinical trial using a colostrum preparation in the management of HIV/AIDS patients presenting with chronic diarrhea. Our results indicate that colostrum-based supplement is effective in reducing the frequency of stool motions and self-estimated fatigue levels, thereby improving a sense of patient well-being and functionality. The intervention group, who in addition to regular therapy received colostrum-based supplement, achieved weight gain and had increased CD4+ cell counts. These findings are consistent with those seen in the previous observational study using colostrum-based supplement [9].

Table 2 Effect of colostrum-based supplement on frequency of diarrhea

	Colostrum-based supplement		Control group		p-value
	N	Stool frequency/day	N	Stool frequency/day	
Baseline	45	7.5 (2.9)	42	6.9 (3.2)	0.376
Week 1	43	3.5 (1.5)	41	4.7 (3.8)	0.059
Week 2	42	2.7 (1.9)	42	3.8 (1.9)	0.009
Week 4	42	1.6 (0.7)	41	2.9 (1.8)	<0.001
Week 9	33	1.3 (1.6)	40	2.7 (3.5)	0.041

Data are as mean (SD)

Table 3 Effects of colostrum-based supplement on self-reported fatigue and weight

		N	Fatigue (VAS score)	*p*-value	*N*	Weight (kg)	*p*-value
Baseline							
	Colostrum-based supplement	45	52.8 (15.3)		45	51.7 (7.7)	
	Control group	42	46.4 (14.8)	0.075	42	54.0 (9.4)	0.140
Week 4							
	Colostrum-based supplement	43	25.1 (11.4)		43	54.1 (6.3)	
	Control group	42	35.5 (11.9)	<0.001	42	54.0 (9.7)	0.927
Week 9							
	Colostrum-based supplement	42	7.9 (8.1)		42	57.6 (6.1)	
	Control group	41	26.6 (8.5)	<0.001	42	53.5 (9.8)	0.024

VAS Visual analogue scale

Data are as mean (SD)

Except for mean age at enrolment, mean CD4+ cell count and mean serum albumin levels, study patients in the two treatment arms were similar in all the other baseline socio-demographic characteristics, symptoms, signs and laboratory findings. The remarkable reduction in the mean frequency of stool of 79% among patients on colostrum-based supplementation at week 4 in this randomized controlled study is comparable to findings in the earlier observational non-randomized study conducted in Nigeria [9]. The percentage reduction in the stool frequency among control patients was lower than that in the intervention group. The prolonged effect of colostrum-based supplement on diarrhea was seen at week 9. The reason for this prolonged effect is not apparent.

Micro-organisms could be identified in only a few stool samples and these were opportunistic infections. We cannot rule out missed diagnosis on microscopy since we did not do stool cultures. However, previous studies have not identified infective etiological agents in most HIV patients with diarrhea. Besides opportunistic bacterial infections, viral infections and HIV enteropathy are major causes of diarrhea in HIV/AIDS patients [11].

Patients on colostrum-based supplement had a lower mean baseline CD4+ counts at enrolment. A selection bias in the allocation of study participants to the treatment groups during randomization process cannot be ruled out. However, in patients who received colostrum-based supplement, the mean CD4+ count increased by 14%; in contrast, the count reduced by 12.2% in the control arm over the 9 week study period. The observational study done in Nigeria [9] documented an increase in the mean CD4+ count of 125% after 4 weeks of ColoPlus treatment.

Weight and body mass index improved in patients in the intervention group; these findings are consistent with those in the earlier study. However, no changes in hemoglobin and serum albumin were found in our study. These indices probably need more time after recovery from diarrhea to show significant improvement.

There are certain limitations to this study. First it was carried out as a single-blind randomized study without an isocaloric placebo arm. We do, however, feel that an addition of a mere 350 kcal (which equals the caloric contents in 100 g of colostrum-based supplement) to the control group would not jeopardize our major conclusions. Also, we did not include an arm with antiretroviral therapy as colostrum-based supplement should act as an adjunct to antiretroviral therapy, not a substitution for antiretroviral therapy. Also, in Northern Uganda antiretroviral therapy was not readily available at the time of the study.

This study thus provides evidence that bovine colostrum, administered as ColoPlus, is effective in treating HIV-associated diarrhea; it also reduces fatigue levels. Patients treated with bovine colostrum achieve greater weight gain, increase in body mass index and show a good immunological response as demonstrated by a rise in CD4+ count. These effects can last for over 1 month after treatment is stopped.

Table 4 Effect of colostrum-based supplement on CD4+ counts

	Baseline		Week 9	
	N	CD4+ counts (/μl)	*N*	CD4+ counts (/μl)
Colostrum-based supplement	45	379.38 (272.40)*	42	432.55 (314.48)
Control group	42	492.02 (281.79)	41	432.20 (253.90) $p=0.996$

Data are as mean (SD)

*$p=0.044$ as compared to control group at baseline

Acknowledgements This work was supported by the Swedish Embassy in Uganda and Gulu Women Economic Development and Globalization, Uganda.

Conflict of interest The authors declare that Claes-Henrik Florén and Lidia Elfstrand are on the advisory board of ColoPlus AB, without up to now getting any financial compensation.

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