

Fecal Calprotectin Concentrations Are Higher in Exclusively Breastfed Infants Compared to Those Who Are Mixed-Fed

Stephanie M. Dorosko,¹ Todd MacKenzie,² and Ruth I. Connor¹

Abstract

Background: Previous studies have shown that giving other foods while breastfeeding increases the risk of mother-to-child transmission of human immunodeficiency virus (HIV) type 1. The mechanism is speculated to be increased inflammation of the gastrointestinal tract.

Methods: In a prospective longitudinal study, we compared fecal calprotectin, a marker of intestinal inflammatory disease, in healthy U.S. infants 0–6 months of age who were exclusively breastfed compared to those given additional liquids or solids.

Results: In comparison to infants who were mixed-fed, fecal calprotectin was significantly higher in the exclusively breastfed group ($p = 0.01$) by a mean of 60 mg/kg (SE = 23).

Conclusions: Introducing complementary food does not increase intestinal inflammation according to this marker. Studies to assess fecal calprotectin levels in breastfeeding and mixed-fed infants born to HIV-infected mothers living in resource-constrained settings, and to evaluate potential protective effects of calprotectin in early infancy, are recommended in the continuing effort to elucidate the mechanisms responsible for increased risk of HIV transmission through mixed-feeding.

Introduction

THE MECHANISM of mother-to-child transmission of human immunodeficiency virus type 1 (HIV-1) through breast-milk is largely unknown. Intestinal inflammation and increased intestinal epithelial permeability of the infant gut resulting from ingestion of pathogen-laden food or water during mixed feeding has been proposed as one explanation.^{1,2} The impact of food antigens on physiologic changes in the intestinal mucosa and subsequent milk-borne HIV transmission is also unknown.

Intestinal biopsy would likely serve as the gold standard to test subclinical intestinal inflammation *in vivo*; however, a stable marker that could be detected in fecal samples would be a welcome surrogate for identifying intestinal inflammation. Calprotectin, a heterodimer also known as S100A8/A9, or myeloid-related protein-8/14, released primarily from neutrophils,³ is detectable in fecal material and extremely stable at room temperature for several days.^{3–5} Enzyme-linked immunosorbent assay (ELISA) techniques have been utilized to measure fecal calprotectin concentrations,^{6,7} and commercial ELISA kits are now available. This protein has increased in popularity as a diagnostic screening marker for inflammatory bowel disease, ulcerative colitis, Crohn's disease, and colorectal neoplasia, because it reliably detects in-

testinal inflammation without the need for invasive techniques.^{8–13}

We set out to determine in a prospective longitudinal pilot study whether mixed-feeding, compared to exclusive breastfeeding (EBF), in a population of healthy U.S. infants was associated with increased subclinical intestinal inflammation as measured by fecal calprotectin.

Subjects and Methods

Study population

Mothers were recruited from the Dartmouth Hitchcock Medical Center (DHMC) Women's Resource Center's Fourth Trimester Support Group. After being informed of the study by the group facilitator or the study coordinator, mothers who volunteered to contribute diapers were asked about their infants' feeding routines according to a specifically designed questionnaire. Information obtained at the time of sample collection included frequency of feeding of breast-milk and infant formula and/or weaning foods, types of formula or weaning food given, and administration of medications and vitamin/mineral supplements. Mothers were encouraged to donate multiple diapers until infants reached 6 months of age.

Departments of ¹Microbiology & Immunology and ²Medicine, Dartmouth Medical School, Lebanon, New Hampshire.

This study was approved by the Dartmouth Medical School Committee for the Protection of Human Subjects.

Methods

Diapers were transported to the laboratory, where up to 2 mL of fecal material was transferred into sterile containers and stored at -70°C . Samples were later thawed at room temperature and analyzed for fecal calprotectin using the Phical ELISA commercial kit (developed by Calpro, Oslo, Norway; manufactured by Eurospital, Trieste, Italy; distributed by Genova Diagnostics, Ashville, NC). Results are expressed in mg/kg. According to the kit manufacturer, the reference median for healthy adults is 25 mg/kg, and values above 50 mg/kg are considered a positive indicator of intestinal inflammation.

Statistical analysis

To examine the association of fecal calprotectin with diet as well as with infant age, we employed a linear mixed-effects model, with fixed effects for age, EBF, EBF with medications/supplements, and mixed-feeding (breastmilk plus infant formula and/or weaning foods). Because of repeated measures, a random intercept was used for each infant. Analysis was carried out using the nlme library of R software (www.R-project.org).

Results

Seventy-seven fecal samples were obtained from 32 healthy term infants 3 days to 6 months old. Nineteen infants were boys, and 13 were girls. The ethnicity of all infants in the study was Caucasian, reflecting the demographics of the region near DHMC.

Forty-one samples were associated with EBF, 15 with EBF and medications/supplements, and 21 with mixed-feeding. Results from one sample obtained from a 6-week-old infant fed only formula (calprotectin concentration 180.23 mg/kg) were not included in the statistical analyses. Medications given to EBF infants within 24 hours of sample collection included simethicone (six samples), acetaminophen (one sample), and ranitidine (one sample). Supplements administered to EBF infants within 24 hours of sample collection consisted of various combinations of vitamins A, C, and D and/or iron (eight samples). One sample was collected from an infant given both vitamin drops and simethicone.

Figure 1 depicts fecal calprotectin concentrations according to age and diet category. From 0 to 6 months, fecal calprotectin was negatively and significantly associated with age ($p = 0.014$), declining on average 14 mg/kg (SE = 6) per month. Mean calprotectin concentration for all samples ($n = 77$) was 198 mg/kg (median = 207, SD = 77, range = 23–359). In comparison to infants who were mixed-fed, and controlling for age, fecal calprotectin was significantly higher in the EBF group ($p = 0.01$) by a mean of 60 mg/kg (SE = 23) and also significantly higher in the group with EBF and medications or supplements ($p = 0.013$) by a mean of 53 mg/kg (SE = 21).

Discussion

We demonstrate in this pilot study of a healthy U.S. infant cohort through 6 months of age that fecal calprotectin

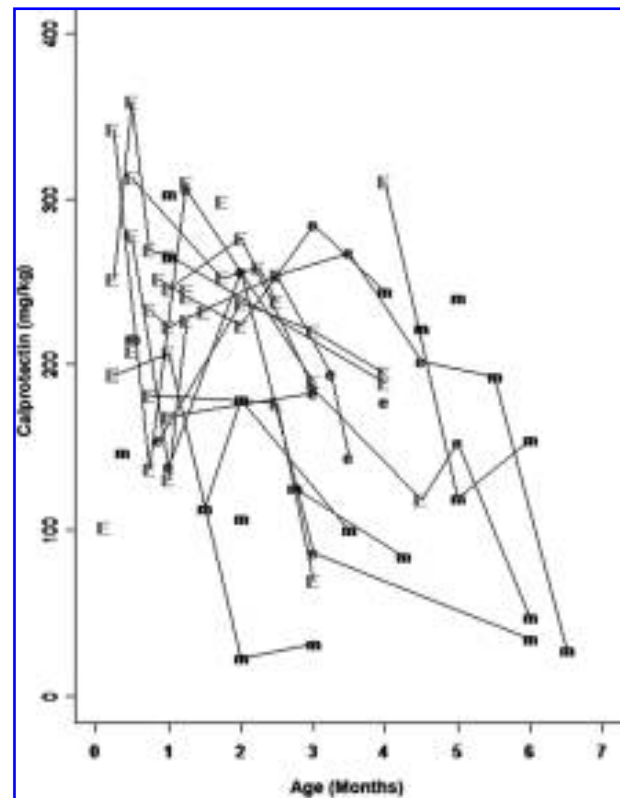


FIG. 1. Fecal calprotectin concentrations (in mg/kg) classified according to infant age (by month) and diet category: EBF (E), EBF with medications or vitamin/mineral supplements (e), or breastmilk plus formula and/or solid food (mixed-fed) (m).

concentrations from EBF infants or EBF infants receiving oral medications and/or vitamin/mineral supplements are higher than those from mixed-fed infants. We do not interpret these findings as increased intestinal inflammation in EBF infants, but rather as a lack of inflammation in mixed-fed infants and perhaps a protective role for calprotectin in young EBF infants. Previous studies utilizing lactulose/manitol ratios found no difference in intestinal epithelial permeability in South African infants who were either EBF or mixed-fed¹⁴ or in a healthy cohort of Spanish infants who were either EBF or given one of four different infant formulas.¹⁵ However, mixed-fed infants who ingest contaminated food or water might have higher fecal calprotectin concentrations than EBF infants.

Our study differs from previous similar studies in that we compared fecal calprotectin from EBF infants to that of infants receiving additional foods or supplements. Other studies have examined age-related fecal calprotectin levels in infants without stratifying according to diet¹⁶ or assessed fecal calprotectin from EBF and weaning (mixed-fed) infants against those who were solely formula-fed.¹⁷ Our study cohort did not have sufficient numbers of non-breastfed infants to include in our analysis. Thus, we are not able to directly compare our results with those from previously published studies.

Elevated concentrations of fecal calprotectin in healthy young infants found in our study as well as in previous stud-

ies of breastfed infants are similar to those of children and adults with inflammatory intestinal pathology.¹⁰ We speculate that calprotectin may serve a protective role for the immature infant intestine, until epithelial tight junction proteins and other factors of intestinal immunity up-regulate with age. Calprotectin has demonstrated both bactericidal and fungicidal properties in vitro,¹⁸ and its presence in the oral mucosa of HIV-infected individuals appears to prevent localized oral candidiasis from further dissemination.¹⁹

The results of this study are unexpected. We hope that this report will foster additional exploration of the relative contributions of ingested and immunologic factors in elucidating the mechanism of HIV-1 transmission across the infant intestinal barrier.

Acknowledgments

Our heartfelt thanks to Suzanne Greeley, R.N., coordinator and facilitator for the DHMC Women's Resource Center's Fourth Trimester Support Group, for informing mothers about the study; to the participating mothers (and infants); and to Stephen Arpad, M.D., Associate Professor of Clinical Medicine, Department of Pediatrics, Columbia University Medical Center, for extremely helpful discussion. This work has been supported by National Institutes of Health Training Grant T32 AI07363, "Immunobiology of Myeloid and Lymphoid Cells," granted to Dartmouth Medical School's Department of Microbiology and Immunology.

References

- Coovadia HM, Rollins NC, Bland RM, et al. Mother-to-child transmission of HIV-1 infection during exclusive breastfeeding in the first 6 months of life: an intervention cohort study. *Lancet* 2007;369:1107-1116.
- Coutsoudis A, Pillay K, Kuhn L, et al. Method of feeding and transmission of HIV-1 from mothers to children by 15 months of age: prospective cohort study from Durban, South Africa. *AIDS* 2001;15:379-387.
- Johne B, Fagerhol MK, Lyberg T, et al. Functional and clinical aspects of the myelomonocyte protein calprotectin. *J Clin Pathol Mol Pathol* 1997;50:113-123.
- Mork G, Schjerven H, Mangschau L, et al. Proinflammatory cytokines upregulate expression of calprotectin (L1 protein, MRP-8/MRP-14) in cultured human keratinocytes. *Br J Dermatol* 2003;149:484-491.
- Hetland G, Talgo GJ, Fagerhol MK. Chemotaxins C5a and fMLP induce release of calprotectin (leucocyte L1 protein) from polymorphonuclear cells in vitro. *J Clin Pathol Mol Pathol* 1998;51:143-148.
- Johne B, Kronborg O, Ton HI, et al. A new fecal calprotectin test for colorectal neoplasia. *Scand J Gastroenterol* 2001;36:291-296.

- Ton HI, Brandsnes O, Dale S, et al. Improved assay for fecal calprotectin. *Clin Chim Acta* 2000;292:41-54.
- Amati L, Passeri ME, Selicato F, et al. New insights into the biological and clinical significance of fecal calprotectin in inflammatory bowel disease. *Immunopharmacol Immunotoxicol* 2006;28:665-681.
- Kapel N, Roman C, Caldari D, et al. Fecal tumor necrosis factor-alpha and calprotectin as differential diagnostic markers for severe diarrhea of small infants. *J Pediatr Gastroenterol Nutr* 2005;41:396-400.
- Olafsdottir E, Aksnes L, Fluge G, et al. Faecal calprotectin levels in infants with infantile colic, healthy infants, children with inflammatory bowel disease, children with recurrent abdominal pain and healthy children. *Acta Paediatr* 2002;91:45-50.
- Bunn SK, Bisset WM, Main MJC, et al. Fecal calprotectin as a measure of disease activity in childhood inflammatory bowel disease. *J Pediatr Gastroenterol Nutr* 2001;32:171-177.
- Bunn SK, Bisset WM, Main MJC, et al. Fecal calprotectin: Validation as a noninvasive measure of bowel inflammation in childhood inflammatory bowel disease. *J Pediatr Gastroenterol Nutr* 2001;33:14-22.
- Roseth AG, Aadland E, Jahnsen J, et al. Assessment of disease activity in ulcerative colitis by faecal calprotectin, a novel granulocyte marker protein. *Digestion* 1997;58:176-180.
- Rollins NC, Filteau SM, Coutoudis A, et al. Feeding mode, intestinal permeability, and neopterin excretion: A longitudinal study in infants of HIV-infected South African women. *J Acq Immune Def Syndr* 2001;28:132-139.
- Colome G, Sierra C, Blasco J, et al. Intestinal permeability in different feedings in infancy. *Acta Paediatr* 2006;96:69-72.
- Rugtveit J, Fagerhol MK. Age-dependent variations in fecal calprotectin concentrations in children. *J Pediatr Gastroenterol Nutr* 2002;34:323-324.
- Golden BE, Bunn SK, Main MJC. Age-dependent variations in fecal calprotectin concentrations in children. *J Pediatr Gastroenterol Nutr* 2002;34:324-325.
- Steinbakk M, Naess-Andresen CF, Lingaas E, et al. Antimicrobial actions of calcium binding leucocyte L1 protein, calprotectin. *Lancet* 1990;336:763-765.
- Eversole LR, Reichart PA, Ficarra G, et al. Oral keratinocyte immune responses in HIV-associated candidiasis. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 1997;84:372-380.

Address reprint requests to:
Stephanie M. Dorosko, DVM, Ph.D.
Department of Microbiology & Immunology
Dartmouth Medical School
1 Medical Center Drive, HB 7556
Lebanon, NH 03756

E-mail: Stephanie.M.Dorosko@dartmouth.edu