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Attribution of Malnutrition to Cause-Specific Diarrheal Illness: Evidence from a Prospective Study of Preschool Children in Mirpur, Dhaka, Bangladesh

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Abstract

We examined whether malnutrition (underweight [WAZ] < -2) increased the risk of diarrhea equally for all enteropathogens. The study was conducted prospectively between January 1999 and July 2002 in Mirpur, an urban slum in Dhaka. Two hundred eighty-nine Bangladeshi children (147 male and 142 female) 2–5 years of age were included in the study. Malnutrition was present in 39% of the children at the time of enrollment. The parents and children were visited and interviewed every other day by health care workers for details about any diarrheal episodes. Stool samples were successfully collected from 62% of episodes of diarrhea. Of the identified enteropathogens, only enterotoxigenic *Escherichia coli* (ETEC), *Cryptosporidium* sp., and *Entamoeba histolytica* were significantly more prevalent in malnourished children. We concluded that the malnutrition attributed risk is not equal for enteric pathogens associated with diarrheal illness.

INTRODUCTION

Diarrheal illness is one of the major causes of morbidity and mortality in children of developing countries.^{1–3} Although the mortality rate resulting from diarrheal illness has been decreased, particularly in Bangladesh, the diarrheal illness-associated morbidity has not changed. The association between malnutrition and diarrheal morbidity has been recognized for decades and is bidirectional.^{2–9} Diarrhea can lead to malnutrition and malnutrition can predispose to diarrhea. It has also been shown that the negative influence of diarrhea on nutrition is enteropathogen specific.³

However, less is known about whether malnutrition contributed diarrheal illness is enteropathogen specific or not. Determining whether the malnutrition-related risk of diarrheal illness varies for different enteric pathogens has implications for the implementation and evaluation of programs designed to improve child health from diarrheal

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diseases. If malnutrition does not increase the risk of diarrhea from all causes equally, intervention programs that improve nutritional status may not have the same potential for reducing children's diarrheal morbidity in areas with different diarrheal diseases profiles. In the present study we tested the hypothesis that the malnutrition-attributed risk for diarrheal diseases is not equal for all enteropathogens.

The study was conducted between January 1999 and July 2002 in Mirpur, an urban slum in Dhaka as previously described.^{8,10-12} Two hundred eighty-nine children (147 male and 142 female), 2–5 years of age, were enrolled. Fifty-six, 83, and 150 children, respectively, were 24–36 months, 37–48 months, and 49–60 months of age at enrollment. All enrolled children and their family members received free primary health care services, including medications, from the project office in Mirpur. Informed consent was obtained from the parents or guardians and the human experimentation guidelines of the U.S. Department of Health and Human Services, the University of Virginia, the Johns Hopkins University Bloomberg School of Public Health, and the Center for Health and Population Research, ICDDR, B, Dhaka, Bangladesh were followed in the conduct of this research.

The parents and children were visited and interviewed every other day by health care workers for details about any diarrheal episodes of the child. Diarrhea was defined as having three or more unformed stools in a 24-hour period. A “diarrheal episode” was defined as being separated from another episode by at least three diarrhea-free days. Children with diarrhea were also detected through their parents contacting project personnel at the field clinic. When diarrheal disease was detected, the child was examined, treatment provided, and a stool sample collected for detailed investigation of enteropathogens, including enteric protozoa. Stool specimens were collected within 24 hours after the reporting of a diarrheal episode. Samples were transported to the ICDDR, B laboratory within 6 hours after collection. From January 1999 to December 2000 stool specimens were transported to the laboratory without transport media. From January 2001, Cary-Blair and buffer glycerol saline (BGS) media were used for transportation of the stool samples.

Anthropometric measurements were taken by trained research assistants at the time of enrollment. Each child was weighed in light clothes with an electronic weighing scale. Malnutrition was defined by weight for age Z-score < -2. Nutritional status was assessed by comparing the weight of the study children with those of NCHS reference population of the same age and gender with the help of Epi-Info 6 version 6.04, Centers For Diseases Control and Prevention (CDC), USA computer package program.¹³ Microbiologic workup of the stool samples was as previously described.¹¹ Isolation of more than one pathogen from the same diarrheal stool specimen was defined as a mixed infection. Pathogen-specific diarrheal morbidity was expressed in incidence rate episode/ child-year. Comparison between two rates was done by the χ^2 test with Fisher's exact correction. Calculation of relative risk, attributable risk, and attributable proportion among the mal-nourished were calculated by Win Episcopy and CIA Software Packages. A Pvalue = 0.05 was considered significant.

Two hundred eighty-nine children, 2–5 years of age, participated in this cohort study, contributing 299,616 person-days of observation. Out of 1,447 reported diarrheal episodes, stool specimens were available for laboratory research in 893 (62%); ETEC and enteroviruses were investigated only in the first year of the study.¹¹ Prevalent pathogens included enterotoxigenic *E. coli* (ETEC), *Aeromonas* spp., *Giardia*, *Cryptosporidium*, and *E. histolytica*.

Children with and without malnutrition were comparable except for their mean age and access to drinking water (Table 1). Malnourished children were younger and had less access to drinking water from the municipal supply.

The relative risk for diarrheal illness was significantly higher in malnourished children, particularly for ETEC, *E. histolytica*, and *Cryptosporidium* (Table 2). No other enteropathogen had a statistically significant association with malnutrition. Attributable proportions among malnourished children were 63%, 47%, and 40% for respectively ETEC, *E. histolytica*, and *Cryptosporidium*. Malnourished children experienced more *E. histolytica*-associated multiple diarrheal episodes (5.45 per 100-child year for malnourished versus 1.76 per 100-child year for non-malnourished $P = 0.004$) and diarrheal episodes with more than one pathogen (17.9 per 100-child year for malnourished children versus 9.04 per 100-child year for non-malnourished children, $P < 0.0001$). We did not find any association between source of drinking water and *E. histolytica*- or ETEC- or *Cryptosporidium*-associated diarrheal illness (data not shown). Similarly, no association was found between the age of the children and diarrheal pathogens except ETEC, which was more common in the younger age group with borderline significance (8.86 per 100-child year versus 3.68 per 100-child year, $P = 0.057$). Stunting (height for age “Z” scores [HAZ] < -2) at baseline also showed a significant association (attributed risk 0.18; 95% CI 0.01–0.35) with *E. histolytica*-associated diarrhea, but not with Cryptosporidia-, ETEC-, and *Giardia*-associated diarrheal illness (data not shown).

Cryptosporidium infection in early childhood has previously been shown to result in malnutrition and associated impairments in cognitive function,^{14–16} and conversely malnourished infants have been shown to be at greater risk for cryptosporidiosis,^{17–19} validating our finding in this preschool age cohort. We had previously reported the higher incidence of *E. histolytica* diarrhea in the malnourished children from this cohort,⁸ and earlier studies had also demonstrated a higher incidence of malnutrition in cases of amebiasis than in controls.²⁰ The ETEC-associated diarrhea was previously shown to have a negative impact on children's growth in the first 4 years of life.^{21,22} Malnourished children from this study were immunocompromised to some extent, because they produced less IFN-gamma (IFN- γ), but more interleukin (IL)-5.²³ This might explain why malnourished children were more prone to Cryptosporidiosis, ETEC, and *E. histolytica*.

We studied diarrheagenic viruses and no association was found between viruses and malnutrition. Kaki and others²⁴ also failed to show association with malnutrition and rotavirus associated diarrhea.¹⁹ Similarly, no association was seen for *Giardia* and malnutrition in another recent study.²⁵

There are several limitations of this study. Children who were malnourished at baseline could have had a different exposure to enteropathogens than children who were adequately nourished. In addition, underweight status at baseline could be the result of previous enteropathogen infections that were not measured in this study and those infections, not the malnutrition *per se*, could be responsible for increased susceptibility to specific enteropathogens via compromised intestinal barrier function. We did not investigate *E. coli* and enteroviruses for the entire 3 years of the study period because of the limitation of resources, and only 62% of diarrheal episodes received an enteropathogen workup. Finally, children with multiple episodes of *E. histolytica* diarrhea may contribute disproportionately to the relative risk and the malnutrition attributed risk for reasons possibly unrelated to malnutrition status. We are conducting a birth cohort study to address these important concerns.

In conclusion, the most important finding of the present study was that malnutrition did not increase the risk of diarrheal diseases caused by different enteropathogens equally. We found that malnutrition significantly increased the risk of ETEC-, *E. histolytica*-, and *Cryptosporidium*-associated diarrhea. The attributable proportion of diarrhea among all and among malnourished children was highest for ETEC, followed by *E. histolytica* and

Cryptosporidium. This finding is of potential public health importance, as it implies that successful nutritional interventions could improve ETEC-, *E. histolytica*-, and *Cryptosporidium*-associated diarrheal incidence.

REFERENCES

1. Bryce J, Boschi-Pinto C, Shibuya K, Black RE. WHO estimates of the causes of death in children. *Lancet*. 2005; 365:1147–1152. [PubMed: 15794969]
2. Petri WA Jr, Miller M, Binder HJ, Levine M, Dillingham R, Guerrant RL. Enteric infections, diarrhea, and their impact on function and development. *J Clin Invest*. 2008; 118:1277–1290. [PubMed: 18382740]
3. Guerrant RL, Oria RB, Moore SR, Oria MOB, Lima AAM. Malnutrition as an enteric infectious disease with long-term effects on child development. *Nutr Rev*. 2008; 66:487–505. [PubMed: 18752473]
4. Mata L. Diarrheal disease as a cause of malnutrition. *Am J Trop Hyg*. 1992; 47:16–27.
5. James JW. Longitudinal study of the morbidity of diarrhoeal and respiratory infections in malnourished children. *Am J Clin Nutr*. 1972; 25:690–694. [PubMed: 4624650]
6. Sepulveda J, Willett W, Munoz A. Malnutrition and diarrhoea: a longitudinal study in among Mexican children. *Am J Epidemiol*. 1988; 127:365–376. [PubMed: 3337088]
7. Black RE, Brown KH, Becker S. Malnutrition is a determining factor in diarrheal duration, but not incidence, among young children in a longitudinal study in rural Bangladesh. *Am J Clin Nutr*. 1984; 39:87–94. [PubMed: 6362391]
8. Mondal D, Petri WA Jr, Sack RB, Kirkpatrick BD, Haque R. *Entamoeba histolytica*-associated diarrheal illness is negatively associated with the growth of preschool children: evidence from a prospective study. *Trans R Soc Trop Med Hyg*. 2001; 100:1032–1038. [PubMed: 16730764]
9. Brown KH. Diarrhea and malnutrition. *J Nutr*. 2003; 133:328S–332S. [PubMed: 12514320]
10. Haque R, Ali IKM, Sack RB, Farr BM, Ramakrishnan G, Petri WA Jr. Amebiasis and mucosal IgA antibody against the *Entamoeba histolytica* adherence lectin in Bangladeshi children. *J Infect Dis*. 2001; 183:1787–1793. [PubMed: 11372032]
11. Haque R, Mondal D, Kirkpatrick BD, Akther S, Farr BM, Sack RB, Petri WA Jr. Epidemiologic and clinical characteristics of acute diarrhea with emphasis on *E. histolytica* infections in preschool children in urban slum of Dhaka, Bangladesh. *Am J Trop Med Hyg*. 2003; 69:398–405. [PubMed: 14640500]
12. Haque R, Mondal D, Duggal P, Kabir M, Roy S, Farr BM, Sack RB, Petri WA Jr. *Entamoeba histolytica* infection in children and protection from subsequent amebiasis. *Infect Immun*. 2006; 74:904–909. [PubMed: 16428733]
13. Hamill PVV, Drizd TA, Johnson CL, Reed RB, Roche AF, Moore WM. Physical growth: National Center for Health Statistics percentiles. *Am J Clin Nutr*. 1979; 32:607–629. [PubMed: 420153]
14. Checkley W, Gilman R, Epstein LD, Saurez M, Diaz F, Cabera L, Black RE, Sterling CR. Asymptomatic and symptomatic cryptosporidiosis: their acute effect on weight gain in Peruvian children. *Am J Epidemiol*. 1997; 145:156–163. [PubMed: 9006312]
15. Checkley W, Gilman RH, Epstein LD, Suarez M, Diaz JF, Cabrera L, Black RE, Sterling CR. Effect of *Cryptosporidium parvum* infection in Peruvian children: growth faltering and subsequent catch-up growth. *Am J Epidemiol*. 1998; 145:156–163. [PubMed: 9006312]
16. Guerrant DI, Moore SR, Lima AA, Patrick PD, Schorling JB, Guerrant RL. Association of early childhood diarrhea and cryptosporidiosis with impaired physical fitness and cognitive function four-seven years later in a poor urban community in northeast Brazil. *Am J Trop Med Hyg*. 1999; 61:707–713. [PubMed: 10586898]
17. Kirkpatrick BD, Daniels MM, Jean SS, Pape JW, Karp C, Littenberg B, Fitzgerald DW, Lederman HM, Nataro JP, Sears CL. Cryptosporidiosis stimulates an inflammatory intestinal response in malnourished Haitian children. *J Infect Dis*. 2002; 186:94–101. [PubMed: 12089667]
18. Adjei AA, Armah H, Rodrigues O, Renner L, Borketey P, Ayeh-Kumi P, Adiku T, Sifah E, Lartey M. *Cryptosporidium* spp., a frequent cause of diarrhea among children at the Korle-Bu Teaching Hospital, Accra, Ghana. *Jpn J Infect Dis*. 2004; 57:216–219. [PubMed: 15507781]

19. Gay-Andrieu E, Adehossi E, Illa H, Garba Ben A, Kourna H, Boureima H. Prevalence of cryptosporidiosis in pediatric hospital patients in Niamey, Niger. *Bull Soc Pathol Exot.* 2007; 100:193–196. [PubMed: 17824314]
20. Wanke C, Butler T, Islam M. Epidemiologic and clinical features of invasive amebiasis in Bangladesh: a case-control comparison with other diarrheal diseases and postmortem findings. *Am J Trop Med Hyg.* 1988; 38:335–341. [PubMed: 2895590]
21. Black RE, Brown KH, Becker S. Effect of diarrhea associated with specific enteropathogens on the growth of children in rural Bangladesh. *Pediatrics.* 1984; 73:799–805. [PubMed: 6374599]
22. Qadri F, Saha A, Ahmed T, Al Tarique A, Begum YA, Svennerholm AM. Disease burden due to enterotoxigenic *Escherichia coli* in the first 2 years of life in an urban community in Bangladesh. *Infect Immun.* 2007; 75:3961–3968. [PubMed: 17548483]
23. Haque R, Mondal D, Shu J, Roy S, Kabir M, Davis AN, Duggal P, Petri WA Jr. IFN- γ production by peripheral blood mono-nuclear cells is associated with childhood resistance to amebiasis. *Am J Trop Med Hyg.* 2007; 76:340–344. [PubMed: 17297046]
24. Kakai R, Wamola IA, Bwayo JJ, Ndinya-Achola JO. Enteric pathogens in malnourished children with diarrhoea. *East Afr Med J.* 1995; 72:288–289. [PubMed: 7555883]
25. Hollm-Delgado MG, Gilman RH, Bern C, Cabrera L, Sterling CR, Black RE, Checkley W. Lack of an adverse effect of *Giardia intestinalis* infection on the health of Peruvian children. *Am J Epidemiol.* 2008; 168:647–655. [PubMed: 18669932]

Table 1

Baseline characteristics of the children with and without malnutrition

Indicator	Without malnutrition	With malnutrition	P values
Mean age \pm SD (in months)	51 \pm 11	47 \pm 12	0.002
Percentage of male children	50 (88/176)	52 (59/113)	0.71
Mother without any education % *	61 (94/153)	69 (68/99)	0.24
Percentage of siblings enrolled	12.5 (22/176)	13.3 (15/113)	0.49
Average family size mean \pm SD	6.18 \pm 2.1	6.5 \pm 2.6	0.32
Average monthly income \pm SD in TK	3711 \pm 3086	4020 \pm 3000	0.73
Cemented house %	25 (38/153)	21 (21/99)	0.51
Drinking water from municipal supply %	91 (139/252)	78 (77/99)	0.003
Percentage of children enrolled during winter months	38 (67/176)	37 (42/113)	0.48

* Total household number 252.

Table 2

Malnutrition attributed cause–specific diarrheal illness in preschool children*

Pathogens	Children without malnutrition				Children with malnutrition				Attributed risk among all (95% CI)	Attributed proportion among malnourished (%)
	Person days	Diarrheal episode	Incidence (episode per 100 child year)	Person days	Diarrheal episode	Incidence (episode per 100 child year)	RR for malnourished children (95% CI)			
<i>Campylobacter jejuni</i>	185802	12	2.36	113814	13	4.17	1.8 (0.81, 3.88)	0.23 (-0.08, 0.52)	44	
<i>Plesiomonas shigelloides</i>	185802	18	3.54	113814	18	5.77	1.6 (0.85, 3.14)	0.19 (-0.06, 0.45)	39	
<i>Shigella flexnerii</i>	185802	20	3.93	113814	14	4.49	1.1 (0.58, 2.26)	0.05 (-0.19, 0.32)	13	
<i>Aeromonas</i> sp.	185802	52	10.22	113814	31	9.94	1.0 (0.62, 1.52)	-0.01 (-0.17, 0.16)	3	
<i>Aeromonas hydrophila</i>	185802	12	2.36	113814	8	2.57	1.1 (0.45, 2.66)	0.03 (-0.27, 0.39)	8	
<i>Aeromonas sobria</i>	185802	10	1.96	113814	10	3.21	1.63 (0.68, 3.9)	0.19 (-0.14, 0.53)	39	
<i>Aeromonas caviae</i>	185802	13	2.55	113814	8	2.57	1.0 (0.42, 2.42)	0.002 (-0.29, 0.35)	0.5	
ETEC	86685	7	2.95	54916	12	7.98	2.7 (1.1, 6.9)	0.40 (0.03, 0.70)	63	
<i>Cryptosporidium</i>	185802	37	7.27	113814	38	12.19	1.7 (1.1, 2.6)	0.20 (0.03, 0.38)	40	
<i>Entamoeba histolytica</i>	185802	32	6.29	113814	37	11.87	1.89 (1.2, 3.0)	0.25 (0.06, 0.44)	47	
<i>Giardia</i>	185802	53	10.41	113814	46	14.75	1.4 (0.96, 2.1)	0.14 (-0.02, 0.30)	29	
Viruses	86685	8	3.37	54916	9	5.98	1.78 (0.69, 4.6)	0.23 (-0.14, 0.58)	44	

* RR = risk ratio; CI = confidence interval; ETEC = enterotoxigenic *E. coli*.