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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 Episcope and CIA Software Packages . A P value ≤ 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 others[24] 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 *Crypstosporidium*-associated diarrheal incidence.

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Table 1

Baseline characteristics of the children with and without malnutrition

Indicator	Without malnutrition	With malnutrition	*P* values
Mean age ± SD (in months)	51 ± 11	47 ± 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 ± SD	6.18 ± 2.1	6.5 ± 2.6	0.32
Average monthly income ± SD in TK	3711 ± 3086	4020 ± 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.

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Table 2

Malnutrition attributed cause–specific diarrheal illness in preschool children*

Pathogens	Children without malnutrition			Children with malnutrition			RR for malnourished children (95% CI)	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)			
Campylobacter jejunii	185802	12	2.36	113814	13	4.17	1.8 (0.81, 3.88)	0.23 (–0.08, 0.52)	44
Plesiomonas shigelloides	185802	18	3.54	113814	18	5.77	1.6 (0.85, 3.14)	0.19 (–0.06, 0.45)	39
Shigella flexnerii	185802	20	3.93	113814	14	4.49	1.1 (0.58, 2.26)	0.05 (–0.19, 0.32)	13
Aeromonas sp.	185802	52	10.22	113814	31	9.94	1.0 (0.62, 1.52)	–0.01 (–0.17, 0.16)	3
Aeromonas hydrophilia	185802	12	2.36	113814	8	2.57	1.1 (0.45, 2.66)	0.03 (–0.27, 0.39)	8
Aeromonas sobria	185802	10	1.96	113814	10	3.21	1.63 (0.68, 3.9)	0.19 (–0.14, 0.53)	39
Aeromonas caviae	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**
Cryptosporidium	185802	37	7.27	113814	38	12.19	**1.7 (1.1, 2.6)**	**0.20 (0.03, 0.38)**	**40**
Entamoeba histolytica	185802	32	6.29	113814	37	11.87	**1.89 (1.2, 3.0)**	**0.25 (0.06, 0.44)**	**47**
Giardia	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.*