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Poor Folate Status Predicts Persistent Diarrhea in 6- to 30-Month-Old North Indian Children[1–3]

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Abstract

Poor micronutrient status is associated with diarrheal illness, but it is not known whether low folate and/or cobalamin status are independent risk factors for diarrhea. We measured the association between plasma folate and cobalamin and subsequent diarrheal morbidity in a prospective cohort study of 2296 children aged 6–30 mo in New Delhi, India. Plasma concentrations of folate, cobalamin, total homocysteine (tHcy), and methylmalonic acid were determined at baseline. Whether a child had diarrhea was recorded during weekly visits in a 4-mo zinc supplementation trial. Diarrhea episodes lasting <7, ≥7, and ≥14 d were classified as acute, prolonged, and persistent, respectively. There was a total of 4596 child periods with acute, 633 with prolonged, and 117 with persistent diarrhea during follow-up. Children with plasma folate concentrations in the lowest quartile had higher odds of persistent diarrhea than children in the other quartiles [adjusted OR = 1.77 (95% CI = 1.14, 2.75); $P = 0.01$]. This effect differed between boys [adjusted OR = 2.51 (95% CI = 1.47, 4.28)] and girls [adjusted OR = 1.03 (95% CI = 0.53, 2.01); P-interaction = 0.030]. We found a small but significant association between high plasma tHcy concentration and acute diarrhea [adjusted OR = 1.14 (95% CI = 1.04, 1.24); $P = 0.006$]. Plasma cobalamin concentration was not a predictor of diarrheal morbidity. In conclusion, poor folate status was an independent predictor of persistent diarrhea in this population. J. Nutr. 141: 2226–2232, 2011.

Introduction

Poor micronutrient status is a risk factor for severe diarrheal illness (1). Although zinc and vitamin A have received the most attention (2,3), folate has also been implicated (1). Tetrahydrofolate are required for DNA and RNA synthesis (4). Because of their intertwined metabolic pathways, a poor supply of either folate or cobalamin can lead to functional intracellular folate deficiency (4). Therefore, tissues with rapid cell turnover, such as the gastrointestinal tract, may be particularly sensitive to poor cellular availability of tetrahydrofolate. However, there is a lack of good studies demonstrating an association between folate and/or cobalamin status and subsequent diarrheal morbidity (1).

Deficiencies of both folate and cobalamin can lead to elevated levels of plasma tHcy, whereas the marker MMA is specific to cobalamin (4). In infancy, however, cobalamin appears to be the main determinant of tHcy (5). We previously reported a high prevalence of poor folate and cobalamin status among 2296 North Indian children aged 6–30 mo and that the plasma concentrations of these vitamins were strongly linked to whether the child was breastfed (6). We also reported that folate, but not cobalamin, status predicts respiratory morbidity in this population (7). These studies were nested within a zinc supplementation trial that yielded substantial reductions in diarrheal morbidity (8). However, whether poor folate or cobalamin status is associated with acute diarrhea or the duration of diarrheal episodes has not been examined. Using data from zinc supplementation trial, we investigated whether poor folate and cobalamin status were predictors for diarrheal morbidity in 6- to 30-mo-old North Indian children.

Participants and Methods

Study setting and participants. The current analysis was performed to address a predefined secondary objective of a zinc supplementation trial in 2482 children aged 6–30 mo (8). The trial took place in the urban slum of Dakshinpuri in New Delhi, India. Children 6–30 mo ($n = 3802$) were

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[3] This trial is registered at www.clinicaltrials.gov as NCT00272116.

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identified through a survey and were not included if their caregivers did not give consent (17.8%), if they were likely to move out of the study area within the next 4 mo (15%), if they needed urgent admission to hospital on the enrollment day (0.5%), or had received a massive dose of vitamin A [100,000 IU (30 mg) for infants and 200,000 IU (60 mg) for older children] within the last 2 mo (1.5%). Of the 2482 children randomized, 96 (3.8%) of the caregivers refused blood sampling, and blood was insufficient for analysis for another 90 (3.6%) children [trial profile shown in (6)]. Daily doses of 10 mg elemental zinc were given to infants (6–12 mo old) and 20 mg were given to older children (12–30 mo old) for 4 mo. Enrollment commenced on February 15, 1998 and the follow-up of the last child was completed on September 30, 2000. The trial was approved by the All India Institute of Medical Sciences ethics committee. Informed written consent was obtained from community leaders and parents. Signatures or thumb impressions were obtained on consent forms and a copy was left with the family.

Outcomes, treatment, and definitions. A study physician interviewed the caretaker, examined the child, and collected a venous blood specimen from the child on enrollment for measuring folate and cobalamin status in addition to zinc and copper. Weight and length (height for children ≥ 24 mo) were measured using Seca Salter scales and locally manufactured length boards that read to the nearest 0.1 kg and 0.1 cm, respectively. Fieldworkers visited the children weekly for the 4-mo study period. At each visit, information was obtained about the number and consistency of stools in the previous 7 d. If the child had diarrhea and vomiting, dehydration was assessed. Two packets of oral rehydration salts were given when a child had diarrhea. Clinical services were available at the study clinic. Children who spontaneously visited the clinic or sick children referred to the clinic by field workers were treated according to WHO guidelines (9). Standardization and quality control procedures were previously described (8).

Diarrhea was defined as passage of ≥3 loose or watery stools in a 24-h period (8) and recovery as the first day of a 72-h period when the child had no diarrhea. Episodes lasting <7, ≥7, and ≥14 d were classified as acute, prolonged, and persistent diarrhea, respectively.

Blood collection and biochemical analyses. Nonfasting venous blood specimens (~5 mL) were collected in heparinized polypropylene tubes (Sarstedt) between 0900 and 1200 h. The samples were centrifuged (447 × *g*, 10 min, room temperature) and the plasma stored in polypropylene vials (Eppendorf) at −20°C until analysis.

Plasma cobalamin (n = 2261) and plasma folate (n = 2296) concentrations were measured by microbiological assays with the use of a chloramphenicol-resistant strain of *Lactobacillus casei* and a colistin sulfate-resistant strain of *Lactobacillus leichmannii*, respectively (10,11). Both assays were adapted to a microtiter plate format and carried out by a robotic workstation (12). Plasma MMA (n = 2270) and plasma tHcy (n = 2271) concentrations were measured with a modified GC-MS method based on ethylchloroformate derivatization (13).

Data management and statistical analyses. The computer entry system for the study was designed with FoxPro for Windows (Microsoft), with range and consistency checks incorporated. Double data entry by 2 data clerks followed by validation and correction of any errors were completed within 48 h of data collection in the field. Anthropometric measures were expressed as Z-scores, which were generated using the WHO Child Growth Standards (14). Stunting was defined as length-for-age Z-score < −2 SD and wasting as weight-for-length Z-scores < −2 SD.

All children for whom baseline venous blood specimens were analyzed for at least one parameter of folate and cobalamin status (n = 2296) were included in the analyses for the present study. The predefined cutoffs for vitamin variables were below < or ≥ the 25th percentile in this dataset for cobalamin and folate, and > or ≤ the 75th percentile for tHcy and MMA (7). We used percentile cutoffs for low or deficient status, because there are no established reference ranges for plasma MMA and tHcy concentrations in infants and because of the uncertainties relating to commonly used cobalamin cutoffs for infants and young children (5).

Continuous variables were reported as means or medians as appropriate and categorical variables as proportions in the baseline table. The 4-mo follow-up period was divided into 17 periods of 7 d each for each child. For a period to be included in the analysis, we required information on ≥4 d of the given 7-d period. We used logistic regression to estimate OR for diarrhea between categories of folate and cobalamin status. To account for the interdependence of multiple observation periods in the same child, the regression models were fitted with generalized estimating equation using the robust option and an exchangeable covariance-variance matrix. Potential confounders were identified through baseline tables stratified by folate, cobalamin, tHcy, and MMA categories. Predictors of diarrhea were identified using a stepwise process (15). Zinc supplementation reduced the incidence of both acute and persistent diarrhea (8) and was adjusted for in these models.

We also assessed potential confounders by adding them to the multiple regression models one at a time. These variables were: age, sex, weight-for-age, length-for-age, and weight-for-length Z-scores, breastfeeding status, season (3 categories), zinc supplementation status, years of schooling of mother and father, maternal literacy, household income, family type, number of household members, self-reported diarrhea 24 h prior to inclusion, and baseline plasma zinc. Based on our previously reported finding that cobalamin status modified the effect of zinc supplementation on the incidence of prolonged diarrhea (16), we examined any interaction of plasma folate, cobalamin, tHcy, or MMA with zinc supplementation status. We also assessed interactions between the variables age, sex, stunting, and wasting and of these variables with folate, cobalamin, tHcy, and MMA on their association with diarrheal illness. A P-value of 0.05 was considered significant.

Statistical analysis was performed with StataIC, version 11. We also categorized children by whether they had ≥1 episode of persistent diarrhea and assessed any nonlinear associations in generalized additive logistic regression models using the statistical software R version 2.0 (17), adjusting for the same variables as in the logistic regression models. Values in the text are mean ± SD or median (25th, 75th percentiles) unless otherwise noted.

Results

Baseline characteristics. The age of the children was 15.3 ± 7.5 mo and 52.5% were boys. Sixty-nine percent of the study population was breastfed. The median (25th, 75th percentiles) plasma concentrations of folate, cobalamin, tHcy, and MMA were 10.7 (6.4, 20.1) nmol/L, 205 (141, 299) pmol/L, 10.9 (8.4, 14.9) μmol/L, and 0.65 (0.37, 1.29) μmol/L, respectively. Children with low (<25th percentile) plasma folate concentrations were somewhat older, a substantially lower proportion of them were breastfed, and they had a much higher plasma cobalamin concentration than children with plasma folate concentrations ≥ 25th percentile. Children with a low plasma cobalamin concentration were slightly younger, a higher proportion was breastfed, and they had higher plasma concentrations of folate, tHcy, and MMA (**Table 1**). Compared with children with lower plasma tHcy concentrations, children with plasma tHcy concentrations ≥75th percentile were younger (mean age 12.6 vs. 16.2 mo), a higher proportion was breastfed (87.5 vs. 62.9%), and they had markedly lower median plasma cobalamin concentrations (129 vs. 233 pmol/L) and higher folate and MMA concentrations (18.9 vs. 9.4 nmol/L and 1.48 vs. 0.52 μmol/L, respectively). Children with plasma MMA concentrations ≥75th percentile were also younger (mean age 12.4 vs. 16.3 mo), a higher proportion was breastfed (94.1 vs. 60.8%), and their plasma folate and tHcy concentrations were higher (19.7 vs. 9.1 nmol/L and 17.0 vs. 9.9 μmol/L, respectively) and cobalamin concentrations lower (136 vs. 229 pmol/L) compared with children with lower MMA concentrations.

Baseline characteristics (age, sex, breastfeeding status, and anthropometric and socioeconomic variables) for the 221 children whose caregivers refused blood sampling or who had insufficient blood for analyses of one or more markers of folate

TABLE 1 Baseline characteristics of children in the study by subgroups of plasma folate and cobalamin concentrations[1]

	Folate (n = 2296)		Cobalamin (2261)	
	<25th percentile[2]	≥25th percentile	<25th percentile[2]	≥25th percentile
n	577	1719	566	1695
Age, *mo*	17.5 ± 7.2	14.6 ± 7.5	14.3 ± 7.1	15.6 ± 7.6
Girls, *n (%)*	265 (46)	826 (48.1)	293 (51.8)	786 (46.4)
Breastfed, *n (%)*	204 (35.4)	1381 (80.3)	518 (91.5)	1045 (61.6)
Length-for-age Z-score < -2, *n (%)*	306 (53.0)	655 (38.1)	247 (43.7)	692 (40.8)
Weight-for-length Z-score < -2, *n (%)*	139 (24.1)	309 (18.0)	106 (18.8)	333 (19.7)
Family income, *1000 rupees*	36 (24, 48)	36 (24, 60)	36 (24, 51)	36 (24, 60)
Schooling, mother, *y*	5 (0, 8)	5 (0, 9)	5 (0, 8)	5 (0, 9)
Enrolled in the hot season, *n (%)*	185 (32.1)	584 (34.0)	196 (34.6)	544 (32.1)
Plasma folate, *nmol/L*	4.7 (3.6, 5.6)	14.0 (9.6, 25.0)	18.8 (11.1, 32.9)	9.1 (5.8, 15.8)
Plasma cobalamin, *pmol/L*	267 (195, 366)	187 (128, 271)	103 (80, 125)	245 (189, 335)
Plasma tHcy, *μmol/L*	10.2 (8.5, 12.8)	11.2 (8.2, 15.9)	16.5 (12.2, 22.4)	9.8 (7.9, 12.6)
Plasma MMA, *μmol/L*	0.42 (0.31, 0.69)	0.75 (0.41, 1.55)	1.14 (0.40, 2.89)	0.53 (0.32, 0.93)

[1] Values are mean ± SD, median (25th, 75th percentile), or *n* (%). MMA, methylmalonic acid; tHcy, total homocysteine.
[2] The 25th percentile for folate was 6.4 nmol/L and for cobalamin 141 pmol/L.

or cobalamin status did not substantially differ from those of the rest of the group (data not shown).

Risk factors for diarrhea. During a total of 250,310 d of follow-up, there was a total of 4596 child periods with acute diarrhea, 633 with prolonged diarrhea, and 117 with persistent diarrhea among children whose baseline venous blood specimens were analyzed for at least one parameter of folate and cobalamin status (n = 2296).

Young age, being enrolled in the hot season (February to May), and self-reported diarrhea in the 24 h prior to baseline were associated with higher odds of both acute and persistent diarrhea. Being breastfed was an important protective factor for persistent diarrhea (**Table 2**). Socioeconomic factors were not associated with acute or persistent diarrhea. The protective effect of breastfeeding on persistent diarrhea was substantially reduced when folate concentration was added to the logistic regression model [adjusted OR = 0.73 (95% CI = 0.46, 1.16); P = 0.18].

Folate and cobalamin status and subsequent occurrence of diarrhea. There was no interaction between any of the markers of folate and cobalamin status and zinc supplementation for acute diarrhea. In crude analyses, low plasma cobalamin and high tHcy and MMA concentrations were associated with subsequent occurrence of acute diarrhea, whereas low folate concentration was not (**Table 3**). After adjustment for potential confounders, these associations were somewhat attenuated, but the association between high plasma tHcy concentrations and acute diarrhea was still significant. The risk estimate for tHcy was not altered after inclusion of plasma folate or cobalamin concentrations in the model (data not shown).

For the association between cobalamin status and prolonged diarrhea, we restricted the analyses to children receiving placebo (n = 1152), because there was an interaction between cobalamin status (plasma cobalamin, tHcy, MMA) and zinc administration. In the placebo group, the OR for prolonged diarrhea were close to 1.0 and not significant for plasma cobalamin, tHcy, and MMA. Plasma folate and prolonged diarrhea were not associated [adjusted OR = 1.06 (95% CI = 0.85, 1.33); P = 0.6].

Zinc administration did not modify the associations between plasma folate, cobalamin, tHcy, or MMA and persistent diarrhea ($P \geq 0.18$); similarly, these associations were not substantially different when restricting the analyses to those receiving placebo. Low plasma folate was a predictor of persistent diarrhea in the crude analyses, but low cobalamin and high tHcy and MMA concentrations were not (**Table 4**). Adjustment, primarily by age, increased the strength of the association between low folate concentrations and subsequent occurrence of persistent diarrhea. Low cobalamin and high tHcy and MMA concentrations remained unassociated with persistent diarrhea. Generalized additive logistic regression models analyses did not reveal any additional nonlinear associations that were not captured by our main regression analysis (data not shown).

Effect modifiers between folate or cobalamin status and diarrhea. We observed a significant interaction between low folate concentration and gender for persistent diarrhea. Thus, low plasma folate concentration was a predictor of persistent

TABLE 2 Predictors of acute and persistent diarrhea in 2296 North Indian children aged 6–30 mo who were followed for 4 mo[1]

	Acute diarrhea		Persistent diarrhea	
	OR (95% CI)	*P*	OR (95% CI)	*P*
Age (per month)	0.98 (0.98, 0.99)	<0.001	0.92 (0.89, 0.96)	<0.001
Breastfed (yes/no)	0.97 (0.88, 1.06)	0.51	0.56 (0.37, 0.83)	0.004
Included February to May	1		1	
Included June to September	0.67 (0.61, 0.73)	<0.001	0.40 (0.26, 0.62)	<0.001
Included October to January	0.75 (0.68, 0.82)	<0.001	0.42 (0.27, 0.65)	<0.001
Self-reported diarrhea[2]	1.34 (1.20, 1.50)	<0.001	1.60 (1.03, 2.49)	0.035

[1] Calculated by logistic regression using generalized estimating equations. Estimate for each variable adjusted for the other variables for which estimates are presented for each particular outcome. Additionally, estimates under acute diarrhea are adjusted for zinc supplementation status, maternal literacy, and family income, and estimates for persistent diarrhea were adjusted for zinc supplementation.
[2] Mother's report of whether child had diarrhea in the 24 h prior to inclusion in the study.

TABLE 3 Associations between concentrations of plasma markers of folate and cobalamin status and acute diarrhea in North Indian children aged 6–30 mo who were followed for 4 mo[1,2]

	Follow-up periods	Periods with episode	OR (95% CI), crude	*P*	OR (95% CI), adjusted[3]	*P*
Folate						
≥25th percentile	26, 768	3, 428	1		1	
<25th percentile	8, 990	1, 163	1.01 (0.92, 1.11)	0.82	1.07 (0.96, 1.19)	0.20
Cobalamin						
≥25th percentile	26, 473	3, 321	1		1	
<25th percentile	8, 770	1, 201	1.11 (1.01, 1.21)	0.025	1.06 (0.97, 1.16)	0.21
tHcy						
≤75th percentile	26, 664	3, 264	1		1	
>75th percentile	8, 757	1, 280	1.22 (1.12, 1.34)	<0.001	1.14 (1.04, 1.24)	0.006
MMA						
≤75th percentile	26, 717	3, 342	1		1	
>75th percentile	8, 687	1, 199	1.12 (1.03, 1.23)	0.012	1.08 (0.98, 1.18)	0.12

[1] Calculated by logistic regression using generalized estimating equations. MMA, methylmalonic acid; tHcy, total homocysteine.
[2] The 25th percentiles for folate and cobalamin were 6.4 nmol/L and 141 pmol/L, respectively, and the 75th percentiles for tHcy and MMA were 14.9 and 1.29 μmol/L, respectively.
[3] Adjusted for age (continuous), whether breastfed (yes/no), season, stunting (length-for-age Z-score < −2), income (log-transformed, continuous), self-reported diarrhea 24 h prior to inclusion, and zinc supplementation (yes/no), as well as repeated observations within each child.

diarrhea in boys but not in girls (**Table 5**). This association was also examined in generalized additive models (**Fig. 1**), where the inverse association between the log OR and plasma folate concentration was linear at folate concentrations < 20 nmol/L (the 75th percentile) in boys. We also observed an interaction between low cobalamin concentrations and breastfeeding for acute diarrhea. The adjusted OR in breast-fed children (n = 1723) was 1.01 (95% CI = 0.92, 1.12); in nonbreast-fed children (n = 759), the corresponding value was 1.48 (95% CI = 1.17, 1.87) (P-interaction = 0.003).

Discussion

In this large cohort of 2296 North Indian children aged 6–30 mo, we found that folate status was independently and significantly associated with the risk of persistent diarrhea in boys but not girls. We also found weak but significant associations between high plasma tHcy and acute diarrhea and between low plasma cobalamin concentrations and acute diarrhea in the nonbreast-fed infants.

It should be noted that this cohort study was carried out within a zinc supplementation trial and thus in a group of children selected for the purpose of assessing the effect of zinc supplementation on infectious disease. If we had designed the study as a cohort study, the target group, and inclusion and exclusion criteria may have been different and the observed associations may also have differed. However, we think that the inclusion and exclusion criteria would have been slightly but not substantially different. Accordingly, the implications for the observed associations would be minor. In addition, any interac-

TABLE 4 Associations between concentrations of plasma markers of folate and cobalamin status and persistent diarrhea in North Indian children aged 6–30 mo who were followed for 4 mo[1,2]

	Follow-up periods	Periods with episode	OR (95% CI), crude	*P*	OR (95% CI), adjusted[3]	*P*
Folate						
≥25th percentile	26, 768	77	1		1	
<25th percentile	8, 990	40	1.55 (1.05, 2.28)	0.026	1.77 (1.14, 2.75)	0.010
Cobalamin						
≥25th percentile	26, 473	96	1		1	
<25th percentile	8, 770	20	0.63 (0.39, 1.01)	0.06	0.62 (0.38, 1.01)	0.06
tHcy						
≤75th percentile	26, 664	87	1		1	
>75th percentile	8, 757	30	1.05 (0.70, 1.58)	0.81	0.91 (0.59, 1.39)	0.65
MMA						
≤75th percentile	26, 717	85	1		1	
>75th percentile	8, 687	32	1.16 (0.78, 1.73)	0.47	1.15 (0.75, 1.75)	0.53

[1] Calculated by logistic regression using generalized estimating equations. MMA, methylmalonic acid; tHcy, total homocysteine.
[2] The 25th percentiles for folate and cobalamin were 6.4 nmol/L and 141 pmol/L, respectively, and the 75th percentiles for tHcy and MMA were 14.9 and 1.29 μmol/L, respectively.
[3] Adjusted for age (continuous), whether breastfed (yes/no), season, stunting (length-for-age Z-score < −2), income (log-transformed, continuous), self-reported diarrhea 24 h prior to inclusion, and zinc supplementation (yes/no), as well as repeated observations within each child.

TABLE 5 Associations between plasma folate concentration and persistent diarrhea by sex[1]

	n	Child periods with episode	OR (95% CI), crude	OR (95% CI), adjusted[2]
Boys				
≥25th percentile	893	36	1	1
<25th percentile	312	28	2.22 (1.35, 3.66)	2.51 (1.47, 4.28)
Girls				
≥25th percentile	826	41	1	1
<25th percentile	265	12	0.92 (0.49, 1.72)	1.03 (0.53, 2.01)

[1] Calculated by logistic regression using generalized estimating equations with interaction term.
[2] Adjusted for age (continuous), whether breastfed (yes/no), season, stunting (length-for-age Z-score < −2), income (log-transformed, continuous), self-reported diarrhea 24 h prior to inclusion, and zinc supplementation (yes/no), as well as repeated observations within each child. *P*-interaction = 0.030.

tion of the zinc supplementation with our exposure variables were carefully considered and accounted for.

The observed association between poor folate status and persistent diarrhea is consistent with the notion that micronutrient status is associated with the severity, rather than the incidence, of diarrheal illness (1). Although persistent diarrhea accounts for only a small proportion of all diarrhea episodes, it accounts, together with dysentery, for 65% of all diarrhea-associated deaths in India (18). Our finding is consistent with our previously reported finding that poor folate status was an independent risk factor for lower respiratory tract infections in these children (7). Together, our findings are important, because they indicate that folate status may affect immune function in this population and they should be followed up by clinical trials.

Many of the common diarrheal pathogens cause damage to the intestinal linings. For example, enteropathogenic *Escherischa coli* and *Cryptosporidium parvum* infections lead to structural changes in the intestinal epithelium and barrier dysfunction, and the extent of these changes is related to the severity of the resulting illness (19,20). Regeneration of the surfaces and recovery may accordingly be dependent on adequate folate status as was shown in a study in mice where folate deficiency led to more severe rotavirus-induced diarrhea in mice (21). Thus, the mechanism for the association between poor folate status and persistent diarrhea could be related to a role of folate in gut integrity or in the innate and/or the adaptive antigen-specific immune response (22–24). It is possible that folate deficiency slows the renewal of epithelial cells in the small bowel mucosa or blunts the immune response, thus delaying recovery from the initial infection. In addition, folate deficiency may render the mucosa more prone to severe injury by common enteric infections.

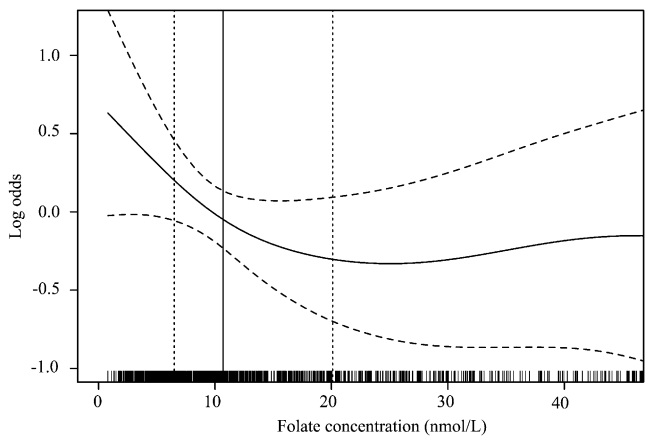

FIGURE 1 Association between plasma folate concentration and odds of having at least one episode of persistent diarrhea in boys aged 6–35 mo (*n* = 1205). The results are adjusted for age (continuous), whether breastfed (yes/no), stunting (length-for-age Z-score < −2), income (log-transformed, continuous), and zinc supplementation status (yes/no). The solid curve depicts the estimated dose-response curve; the dashed curves represent the 95% CI. The vertical lines depict the 25th, 50th, and 75th percentiles for plasma folate concentration of boys and girls combined. The small lines on the *x* axis show the distribution of the observations. The graph was made by using generalized additive models in R (17).

There are few studies reporting on the association between folate or cobalamin and diarrhea in children. In a RCT among 6- to 23-mo-old Bangladeshi children with acute diarrhea, there were no differences in diarrheal duration or stool output between children receiving folic acid (5 mg at 8-h intervals for 5 d) or placebo (25). Provision of a snack to 5- to 12-y-old Columbian school children was associated with increased plasma cobalamin concentrations and fewer reported days with morbidity symptoms including diarrhea (26). However, the intervention was not randomized and the effect on morbidity could also be related to increased energy intake or other nutrients in the snack.

Reverse causality could be an alternative explanation for our results, i.e. that poor folate status was a consequence of a high burden of diarrheal disease prior to enrollment into the study. A diarrheal infection can lead to decreased dietary intake (27) as well as malabsorption of dietary folate (28) at a time when the requirement for folate by the immune system and the gastrointestinal tract is likely to be higher than normal (29). This adverse effect on folate status could in turn render the child more susceptible to new infections and to acute infections becoming persistent, and it may be that children with high prior diarrheal disease burden were the ones that developed persistent diarrhea during the study period. Recent diarrheal illness was associated with subsequent persistent diarrhea in a matched case-control study in India (30). Our only measure of recent illness was mothers' reports of diarrhea 24 h prior to inclusion in the study. However, the proportions with self-reported diarrhea in the past 24 h before inclusion were similar among children in the low- and reference folate groups and inclusion of this variable in multivariate analyses did not change our results. In addition, the most important predictor of folate status was whether a child received breast milk, an important source of folate for young children (31). Therefore, it is likely that the folate deficiency associated with persistent diarrhea in this study was related to dietary intake. Should, however, infection be the cause of poor folate status, folic acid supplementation may be necessary to break the cycle of defective folate uptake and infections.

Breast milk is a good source of folate and the folate concentration in breast milk is not affected by the mothers' folate status (31,32). In our study, breast-fed children had nearly one-half the odds of persistent diarrhea compared with those who were not, a finding corroborating that of earlier studies (33–35). Adjustment for plasma folate substantially reduced the association between breastfeeding and persistent diarrhea. This indicates that the protective properties of breast milk could in part be due to its folate concentration. A similar pattern was observed when we explored the association between folate status and pneumonia (7).

Our subgroup analyses revealed that the association between poor folate status and persistent diarrhea was evident in boys only. Differential effects of micronutrient supplementation on mortality, dysentery, and pneumonia have been observed for boys and girls (36–39). In our study, the difference in plasma folate between boys and girls was small (median 10.6 vs. 11.0 nmol/L) and did not change according to folate concentration category. Furthermore, the incidence of persistent diarrhea was similar in boys and girls (64 in boys vs. 63 in girls). We can accordingly not explain the differences in persistent diarrhea by sex or folate status, and further research on this observed gender difference is needed.

Our finding that children in the highest quartile of plasma tHcy concentrations had significantly higher odds of acute diarrhea compared with children with normal plasma tHcy could be independent of vitamin status. Indeed, plasma tHcy increases with activation of the immune system (40). Neither plasma folate nor plasma cobalamin were independent predictors of acute diarrhea, and the risk estimate for tHcy was not altered after inclusion of plasma folate or cobalamin concentrations in the model. This is consistent with previous findings, which showed that plasma tHcy and folate status predicted acute respiratory tract infections independently of each other (7). Therefore, immune activation may explain the association between higher tHcy at baseline and subsequent episodes of acute diarrhea. It should also be noted that our sample size was large, which increases the probability of a small difference becoming significant.

The interaction between cobalamin status and zinc supplementation for prolonged diarrhea has been discussed elsewhere (16). Despite the poor cobalamin status of this population, plasma cobalamin was not associated with the occurrence of diarrhea in the placebo group, consistent with our finding of no association between low plasma cobalamin levels and risk of subsequent acute lower respiratory tract infection or clinical pneumonia (7). However, among nonbreast-fed children, we observed that those with low plasma cobalamin concentrations had >50% higher odds of acute diarrhea compared with children with normal plasma cobalamin. Thus, it seems as if the protective effect of breastfeeding may outweigh the potential negative consequences of low plasma cobalamin status with regards to acute diarrhea. Another possibility is that we did not have sufficient statistical power to detect a difference of <50% between the exposure categories for persistent diarrhea. Finally, a recent study suggests that low cobalamin status in breast-fed infants is difficult to assess using serum cobalamin measurements (41).

A strength of this study is that it is based on a large, representative sample of a well-defined, low-income community. However, we cannot rule out the possibility that the observed associations are confounded by variables we did not measure or that there could be limitations in relation to measurements of folate and cobalamin status (42). In conclusion, poor folate status was an independent predictor of persistent diarrhea among urban North-Indian male children aged 6–30 mo. Some of the protective effect of breastfeeding on persistent diarrhea may be explained by its folate content. Our findings warrant further exploration in clinical intervention trials.

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