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Helicobacter pylori Infection in the Young in Bangladesh: Prevalence, Socioeconomic and Nutritional Aspects

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Background. The gastric acid barrier, an important host defence against small bowel infection, may be compromised by infection with *Helicobacter pylori*. In developing countries, *H. pylori* infection occurs early in life and prevalence of hypochlorhydria is high particularly in the malnourished, which may predispose a child to repeated gastrointestinal infection and diarrhoea. Diarrhoea being a leading cause of childhood mortality and morbidity in developing countries, we investigated the prevalence of *H. pylori* infection in children in a poor Bangladeshi community and explored its association with socioeconomic and nutritional status.

Methods. The study was conducted in a poor periurban community among 469 children aged 1–99 months. Parents were interviewed using a questionnaire To detect active infection with *H. pylori* a ^{13}C-urea breath test was performed and weight was recorded on a beam balance with a sensitivity of 20 g.

Results. In all, 61% of 36 infants aged 1–3 months were positive for *H. pylori*; this rate dropped steadily with increasing age and was 33% in 10–15 month old children and then rose to 84% in 6–9 year olds. Overall *H. pylori* infection had no association with nutritional state of the child, or family income but the infection rate was 2.5 times higher in children of mothers with no schooling.

Conclusions. The *H. pylori* infection rate is very high in early infancy in a poor periurban community of Bangladesh. The reason for a drop in the infection rate in late infancy is unclear but could be due to initial clearance of the infection by the body's defence mechanisms but with possible alteration of the gastric mucosa which sustains infection. Maternal education may be protective and may operate through some unidentified proximate behavioural determinants. The rate of *H. pylori* infection in infants and young children may predispose them to repeated gastrointestinal infection and diarrhoea.

Keywords: *Helicobacter pylori*, infants, nutrition, socioeconomic status, diarrhoea, developing countries

Helicobacter pylori is an important cause of chronic active gastritis and plays an important role in the aetiology of peptic ulcer disease in humans. It may be acquired at any age but once acquired, the infection persists for years and often for a life time. The age-specific prevalence of *H. pylori* infection is higher in developing countries than developed countries.[1] Within a country age-specific prevalence is higher in lower socioeconomic groups. Recent data from Lima, Peru[2] demonstrated a direct association between the prevalence of *H. pylori* infection and source of drinking water. The results of a study in The Gambia[3] suggests, for the first time, a close association between *H. pylori* infection and chronic diarrhoea with malnutrition or with severe malnutrition (without diarrhoea) in children. Persistent diarrhoea is an important cause of diarrhoea associated and overall mortality in children of developing countries.[4] The gastric acid barrier, an important host defence against small bowel bacterial contamination,[5,6] may be compromised in infection with *H. pylori*.[7–9] In developing countries *H. pylori* infection occurs early in life[2,3,10,11] and the prevalence of hypochlorhydria is reported to be high particularly in the malnourished,[6,12–16] which may predispose a child to repeated gastrointestinal infection and persistent diarrhoea. Diarrhoea being a leading cause

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of childhood mortality and morbidity in developing countries, we have investigated the prevalence of *H. pylori* infection in infants and children in a poor periurban community in Bangladesh using the ^{13}C-urea breath test and explored its association with nutritional state and socioeconomic factors.

METHODS

Study Population

The study was carried out in a periurban village named Nandipara, 10 km north-east of Dhaka and settled by people of low socioeconomic status on government land. The following information is based on an earlier census of this population (Akramuzzaman S, Mitra A, Mahalanabis D—unpublished). The estimated population was 3000 with about 500 children under 5 years living in a 2.5 square mile area. This population is served by a weekly clinic run by the International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR, B). Among the dwellers, 70% of heads of households were day labourers, 20% were rickshaw pullers and 5% were carpenters or service holders or small businessmen; for the remaining 5% information was not available. Women were mostly housewives (85%); the rest worked as day labourers or helpers in houses or did construction work. Most families live in poorly constructed houses. The study was done in the dry, cooler months between January and March. The village is divided into five neighbourhood population clusters separated by low agricultural land and ponds. During the hot, humid, rainy season, the area is surrounded by water and is cut off from the city road; boats are used to travel to and from the village for about 4 months a year (June–September). Water for drinking and cooking is usually fetched from tubewells or supplied by the City Corporation. Water from nearby ponds and ditches is used for washing utensils, bathing, and cleaning purposes. Sixty per cent of the population have access to sanitary latrines but their use, particularly by children, was very low. Most children defecate in open spaces, a potential source of contamination of the environment. Infants and children over a wide age range 1–99 months were studied. To record information about the child, family and socioeconomic status, a questionnaire was developed and tested by administering it to parents of 10 children not included in the study. An experienced senior health worker from the team working for the hospital-based surveillance study[17] administered the questionnaire. One of us (MMR) examined and weighed the child on a beam balance (Seca) with a sensitivity of 20 g and administered the ^{13}C-urea breath test. This was performed to detect the presence of *H. pylori* infection in the stomach as described below.

^{13}C-Urea Breath Test[18]

In the presence of the enzyme urease orally administered urea is hydrolyzed into ammonia and carbon dioxide. If the urea carbon is labelled with the stable isotope ^{13}C then it can be detected as labelled carbon dioxide in a breath sample. *Helicobacter pylori* is the commonest urease producing gastric pathogen and therefore a positive urea breath test can generally be equated with the presence of a *H. pylori* infection. The urea breath test has been shown to be very robust with a high degree of specificity and sensitivity. This non-radioactive non-invasive test has been successfully used as a diagnostic tool for *H. pylori* infection and was also found useful in children.[2] After obtaining a baseline breath sample in a vacutainer tube (Beckton Dickinson, New Jersey, USA) following a 2-hour fast, a test dose of ^{13}C-urea (99% ^{13}C-urea, Tracer Technologies, Boston, USA) at a dose of 30 mg for children <24 months, 40 mg for children >24 months, was administered along with a liquid meal in order to delay gastric emptying and another breath sample was collected at 30 minutes. Breath samples were collected through a two-way paediatric mask with attached non-return inlet valve into a vacutainer tube in duplicate and shipped to Basel (Switzerland). ^{13}C carbon dioxide was estimated by automated gas-isotopic ratio mass spectrometry at the Department of Medicine and Research, University Hospital, Basel. An increase in the ratio of ^{13}C carbon dioxide in the breath samples after the test dose of ^{13}C-urea compared to that in the fasting state is indicative of a positive test. A cutoff point of 5 in the difference in ratio was used.

RESULTS

In all 469 infants and children aged 1–99 months were studied. Of the mothers, 369 (79.2%) had no schooling; 61 (13.1%) mothers had 1–5 years of schooling and only 36 (7.7%) had ≥6 years of education. Median family income was £33 per month and 88% had a family income of ≤£50 per month. Of the children, 316 (67.4%) were receiving some breast milk. Twenty children (4.3%) were severely malnourished (i.e. <60% weight for age) and another 215 (45.8%) were undernourished (weight for age 60–74%) (Table 1).

The proportion of infants and children (excluding those who had received antimicrobials during the 6 weeks prior to test) positive for *H. pylori* by ^{13}C-urea test are shown in the Figure. Including children who received antibiotics gave similar results. The results

TABLE 1 *Characteristics of 469 children from a semirural poor community in Bangladesh tested for* Helicobacter pylori *by* ^{13}*C-urea breath test*

Median age in months (range)	42 (1–99)
Sex, male/female	238/231
Number of mothers without any formal school education	269 (78.7%)
Median numbers of people living in the same room (range)	4 (2–11)
Presently receiving breast milk	316 (67.4%)
Weight for age (% NCHS[a] median)	
<60%	20 (4 3%)
60–74%	215 (45.8%)
>75%	234 (49.9%)

[a] National Centre for Health Statistics

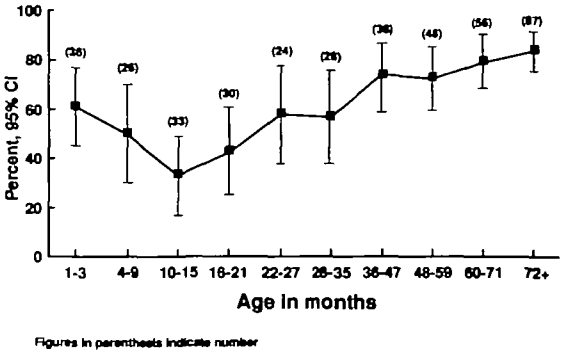

FIGURE *Proportion of 406 children positive for* ^{13}*C-urea breath test for* H. pylori. *Sixty-three children with a history of taking antimicrobials during the previous 6 weeks were excluded. The curve (not shown) including these 63 children was similar to the above*

of the ^{13}C-urea breath were clear cut; ^{13}C-carbon dioxide borderline results (i.e. increase in the ratio of ^{13}C-carbon dioxide to ^{12}C-carbon dioxide close to the cutoff point) were rare. In the very young, infants aged 1–3 months (n = 36), 61% were positive for *H. pylori*; this rate dropped steadily with increasing age to 33% in the 10–15 month age group. From the age of 16 months onwards the rate of positive tests rose steadily and in children aged 6–9 years 84% were positive for *H. pylori*.

The nutritional status of those with positive breath tests was comparable to those who were negative for *H. pylori* in five age groups (Table 2). Breastfeeding was universal in all infants and young children <3 years (97% were breastfed). Sixty-three children had taken some antimicrobials during the 6 weeks prior to the breath test. The antimicrobials taken included (in order

TABLE 2 *Factors associated with* Helicobacter pylori *infection* (^{13}*C-urea test) in a group of children from a periurban poor community in Bangladesh*

Mean ± SD weight for age as % NCHS median[a] (No.)	^{13}C-urea breath test				
	Positive		Negative		
	Mean ± SD (No.)		Mean ± SD (No.)		*P*-value
Age:					
1–9 months	85 ± 15 (36)		90 ± 13 (32)		0.17
10–21 months	75 ± 12 (29)		75 ± 10 (43)		0.90
22–35 months	75 ± 8 (38)		77 ± 11 (26)		0.30
36–59 months	75 ± 8 (78)		77 ± 7 (29)		0.27
60–99 months	74 ± 9 (133)		74 ± 10 (25)		0.90
Presently receiving some breast milk	Yes	No	Yes	No	
1–3 months	22	1	15	0	0.61*
4–35 months	75	5	86	0	0.024*
36–59 months	63	14	25	4	0.59*
⩾60 months	27	105	1	24	0.049*
Took antimicrobials during the previous 6 weeks	42	272	21	134	

[a] t-test, NCHS: National Centre for Health Statistics
* Fisher's exact test (2-tailed).

of frequency) pivmecillinam, tetracycline, nalidixic acid, erythromycin, cloxacillin, penicillin, amoxicillin and cotrimoxazole. In the 1–11 month age group antibiotic use was associated with a significantly reduced rate of positive breath test (11% versus 52%, $P = 0.031$, Fisher's exact test, 2-tailed); however in 12–23 month (60% versus 42%, $P = 0.32$) and in >2 year age groups (77% versus 76%, $P = 0.92$) no significant difference in the rate of positive tests was seen.

Finally logistic regression analysis was carried out to evaluate the association of maternal education (a socioeconomic status indicator) and nutritional state with *H. pylori* infection (Table 3) after adjusting for several confounding factors. In this model the children of mothers with no school education had nearly 2.5 times higher risk ($P < 0.001$) of being positive for *H. pylori*. However, undernutrition was not shown to be associated with the presence of *H. pylori* infection (odds ratio = 1.23, $P = 0.34$). These results were adjusted for age (categorized into four groups), sex, crowding (people living in the same room), low income (i.e. less than the median family income), use of antimicrobials during the previous 6 weeks and lack of breastfeeding. Because of the non-linear relationship between age and infection rate, age categories were based on the

TABLE 3 *Association of nutritional state (wt/age %) and maternal education (a socioeconomic indicator) with positive* Helicobacter pylori *breath test (dependent variable) in 464 children: Logistic regression analysis*[a]

Variables	Regression coefficient	Standard error	*P*-value
Maternal education (no formal education = 1, ≥1 year of formal schooling = 0)	0.880	0.256	0.0006
Undernourished (wt for age as % of NCHS[b] median <75 = 1, else = 0)	0.210	0.221	0.3417

[a] Adjusted for age (categorized into <3 m, 4–22 m, 48+ m), sex, people living in the same room, low income (i.e. < median family income), use of antimicrobials during previous 6 weeks, and lack of breastfeeding. Age categories were based on *H. pylori* infection rate by age and three design variables were created to adjust for its confounding effect.
[b] National Centre for Health Statistics.

distribution of infection rate by age and dummy variables were created to adjust its confounding effect.

DISCUSSION

To understand how common *H. pylori* infection is in infants and young children in developing countries we looked for evidence of active infection using the ^{13}C-urea breath test in a group of 469 children aged one month to 8 years. We have also examined its association with nutritional status, age and socioeconomic indicators (i.e. maternal education and family income). A very high infection rate in infants aged 1–3 months was shown; the infection rate appeared to drop steadily to 10–15 months of age then rose steadily to reach the level of early infancy by 28–35 months. The rate continued to rise thereafter to reach a very high level by the age of 8 years (more than 80%). The reason for this phenomenon is unclear but we speculate that the body's defence mechanism may be able to clear the initial infection but it may leave behind altered conditions (e.g. gastritis, damaged mucosa) which facilitate and sustain subsequent infection with *H. pylori*. No supporting data are available for this speculation but this report should stimulate further pathophysiological and experimental studies in this area. Caution is necessary in interpreting these results because we only looked for point prevalence; longitudinal studies in infants are necessary to confirm that clearance of infection occurs followed by re-infection. This relationship was not altered whether we included or excluded children who had a history of taking antimicrobials in the recent past (Figure). We were able to confirm antimicrobial use in these children from the weekly clinic records of the Centre. Eleven infants were only one month old and eight were positive (73%); 16 were 2 months old, of whom 10 were positive (62%) and 12 were 3 months old of whom 5 were positive (42%).

The ^{13}C-urea breath test can be easily applied to infants, even those as young as one month and gives a clear cut result i.e. the difference in the ratios (^{13}C-carbon dioxide/^{12}C-carbon dioxide) either clearly positive or clearly negative. Human tissue does not contain a urea splitting enzyme. The only known urease producing bacteria in the stomach is *H. pylori*. While some oral bacteria may be able to split urea, a breath sample taken at 30 minutes or later is known to eliminate the possibility of oral bacteria giving a positive breath urea test. It should be pointed out that this test in infants has not yet been validated against the gold standard of gastric biopsy for obvious ethical reasons. Given our present knowledge it is most unlikely that a positive breath test could be due to a cause other than *H. pylori* infection. A negative result due to an inadequate breath sample was unlikely because we discarded samples which did not contain native carbon dioxide to indicate that it was an expired breath sample.

Helicobacter pylori infection is transmitted from person to person by ingestion; both faecal-oral and oral-oral transmission have been postulated.[19–21] Although there is no known environmental reservoir of *H. pylori* a higher rate of infection was seen in Peru in people using the municipal water source compared to those having private wells.[2] A recent seroprevalence study in Chile has shown an association between *H. pylori* infection and consumption of uncooked vegetables; in Chile water for irrigation is polluted by raw sewage.[22] The *H. pylori* infection rate in the very young was exceptionally high in this poor Bangladeshi community. A contaminated environment, crowding, lack of proper sanitation and lack of a sufficiently clean water supply may all explain these findings.

In this population *H. pylori* infection was not associated with poor nutritional state as measured by weight-for-age. These results are at variance with those reported by Sullivan *et al.* in their Gambian study.[3] The odds of having *H. pylori* infection was 14 times higher in children with severe malnutrition without diarrhoea (our calculation of the Gambian data). In the Gambian study however, no adjustment was made for confounding effects e.g. age. The association between lack of breastfeeding and *H. pylori* infection could not be tested because infants and children under 3 years were nearly all breastfed. Although an association (significant at 5% level) was shown between breastfeeding and

H. pylori infection in children over 5 years old, the significance of this finding is tenuous and could be attributed to the effect of multiple comparison. One other interesting finding was that even in this very poor community an association could be shown between *H. pylori* infection and an important socioeconomic indicator (i.e. maternal education). Maternal education as a protective factor may operate through some proximate determinants which are likely to be behavioural in nature. The lack of association between family income and *H. pylori* infection may be due to the fact that the study group is relatively homogenous for family income and sufficient variability in income is lacking.

Infants and young children with *H. pylori* infection are liable to have gastritis and hypochlorhydria and are therefore more susceptible to repeated gastrointestinal infection with faecal coliforms and/or enteropathogens. This may offer, albeit partially, an explanation for the high incidence of diarrhoea in children in Bangladesh. An earlier unpublished longitudinal study on morbidity from various illnesses in 400 children from this study area also documented a high incidence of diarrhoea. These results indicate that cohort studies, e.g. with a newborn cohort, should be carried out to determine the role of early *H. pylori* infection in making infants and children more susceptible to diarrhoea.

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