Helicobacter pylori Colonization in Infants and Young Children is Not Necessarily Associated with Diarrhoea

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Summary

A cohort of 151 infants and young children aged 1–23 months from a poor peri-urban community of Bangladesh was studied to determine the relationship between *Helicobacter pylori* colonization and morbidity due to diarrhoea. A ^{13}C urea breath test was performed to detect the presence of *H. pylori*. Children were followed up at home every alternate day for 6 months and diarrhoeal morbidity data were collected. Diarrhoeal morbidity was compared between *H. pylori*-positive and *H. pylori*-negative children. Sixty-eight (45 per cent) children were *H. pylori* positive and 83 (55 per cent) were *H. pylori* negative. During the first 1-month period following the breath test, three (4.4 per cent) *H. pylori*-positive and four (4.8 per cent) *H. pylori*-negative children had diarrhoea. Thirty-two (47 per cent) of the children in the positive group and 43 (52 per cent) in the negative group had one or more episodes of diarrhoea during the 6-month follow-up period. Median number of diarrhoeal episodes was 1.0 (range 1.0–4.0) in the *H. pylori*-positive children and 2.0 (range 1.0–5.0) in the *H. pylori*-negative children ($p = 0.19$). No significant difference was observed in the cumulative days with diarrhoea. The results of this study suggest that *H. pylori* colonization is not associated with diarrhoeal morbidity in infants and young children.

Introduction

Low gastric acid production has been found to be associated with an increased risk of enteric infection in developing countries.[1,2] There is evidence that acute *H. pylori* infection causes hypochlorhydria in humans.[3,4] It is therefore possible that *H. pylori*-associated hypochlorhydria may lead to increased susceptibility to enteric pathogens. In fact *H. pylori* has been shown to be associated with chronic diarrhoea and malnutrition.[5]

In developing countries, *H. pylori* colonization is acquired in infancy and in early childhood.[6-8] In Peru, 25–50 per cent children under 2 years of age were found to be *H. pylori* positive.[6] In India 60 per cent of 3–10-year-old children were found to be positive for *H. pylori*.[8] Preliminary studies utilizing a ^{13}C urea breath test in a semi-urban Bangladeshi population indicate that more than 60 per cent of children under 6 months of age are also *H. pylori* positive.[9] Thus it seems that in the least developed countries, *H. pylori* colonization may be acquired early in life. However, little is known about whether this high prevalence of *H. pylori* colonization is associated with increased risk of diarrhoeal illnesses. To evaluate this we carried out a cohort study in a peri-urban poor community of Bangladesh.

Study Area

This study was conducted in a peri-urban community adjacent to the capital city of Bangladesh from January

to October 1993. The community consists of a population of 3000 in an area of 2.5 square miles. Among the dwellers, 70 per cent of household heads were day labourers, 20 per cent were rickshaw pullers, and 5 per cent were carpenters or service-holders or small traders. Women were mostly housewives (85 per cent); the rest worked as day labourers. Most of the houses in this community are mud-walled with a thatched or bamboo roof. Seventy-five per cent of the households comprise a single-room house. The community has a water supply from the municipality, which is used mainly for drinking and cooking. For bathing and washing they use water from ditches and ponds. Sixty per cent of the people have access to sanitary latrines. However, the rate of use of these latrines, particularly among children, is low; they usually defaecate in open spaces, which may be a potential source of contamination of the environment. As a whole this is a very densely populated community with poor environmental sanitation. In this community, a weekly clinic is run by the International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR, B) where free medical services are offered.

Subjects and Methods

Infants and children aged 1 to 23 months of age were recruited for this study after parental consent. All children were free from systemic infection causing diarrhoea, respiratory infection, and others at the time of enrolment. The child was examined by a physician and baseline history (age, feeding status, and socioeconomic information) was obtained. A urea breath test (UBT) was performed to diagnose the presence of *H. pylori* infection in these children. These infants and children were visited at home every alternate day to ascertain diarrhoeal morbidity by trained health assistants for a period of 6 months. Diarrhoea was defined as passage of three or more watery/liquid stools in 24 h or bloody mucoid stool. During follow-up the sick children were given treatment at the weekly clinic. Once diagnosed, the watery diarrhoea was treated with oral rehydration solution (ORS) and shigellosis was treated with nalidixic acid. Weight and height were recorded each month.

Breath urea test

The ^{13}C urea breath test (UBT) has been described elsewhere.[9-11] Briefly, a baseline breath urea sample of expired air was collected (0 h) into a Vacutainer (Becton Dickinson, New Jersey, USA) using a mask with non-return inlet valve and septum. The child was then fed 100 ml of milk. Half an hour later, ^{13}C urea (99 per cent ^{13}C, Tracer Technologies, Boston, USA) was given in a dose of 30 mg. After administration of the ^{13}C urea, breath samples were again collected after 30 min and were stored for shipment to the University of Basel, Switzerland to measure ^{13}C concentration of $^{13}CO_2$ in breath samples by mass spectrometry. The excess over the baseline value of $^{13}CO_2$ was expressed as parts per thousand (Δ per cent). A cut-off point of 5 in the ratio was regarded as positive for *H. pylori* colonization.

The ^{13}C urea breath test has been validated in older children as a non-invasive, reliable method for detecting *H. pylori* infection.[10-12] As shown by Vandenplas *et al.* 1992,[12] the sensitivity and specificity of the UBT were 96 and 93 per cent respectively, taking culture as the 'gold standard', and the positive and negative predictive values were 83 and 99 per cent respectively. This test has not been validated in infants under 6 months because

TABLE 1
Baseline characteristics of the children studied
(n = 151)

Variable	H. pylori-positive (*n* = 68)	H. pylori-negative (*n* = 83)
Age (months)		
1–5/6–11/12–23, *n*	30/13/25	28/23/32
Mean ± SD	8.9 ± 7.0	9.9 ± 6.7
Sex (M/F	33/35	58/25
Breastfed (yes/no)	62/6	83/0
Weight for age (Z score)		
< −2.0/ − 2.0 to −1.0/ − 1.0+. *n*	25/21/22	30/26/27
Mean ± SD	−1.6 ± 1.3	−1.6 ± 1.1
Family size		
≤4/5–6/ ≥ 7, *n*	21/28/19	35/36/12
Mean ± SD	5.8 ± 2.2	5.2 ± 2.1
Number of persons living in one room		
3/4–5/ ≥ 6, *n*	22/33/13	34/41/8
Mean ± SD	4.4 ± 1.4	4.0 ± 1.2
Family income ($ US/month)		
≤40/40–75/ > 75, *n*	14/46/8	17/54/12
Mean ± SD	60.0 ± 42.0	66.0 ± 36.0

invasive tests such as gastric biopsy are not justified ethically. However, ^{13}C urea breath test, stool examination by polymerase chain reaction (PCR), and serology to detect *H. pylori* were compared recently in Bangladeshi children aged 4–24 months. When compared with PCR the sensitivity of UBT was >80 per cent (Sarker *et al.* 1997, data submitted to European Society for Paediatric Gastroenterology and Nutrition meeting, 1997; personal communication).

Data analysis

Data were checked and analysed with the SPSS PC+ statistical package (Chicago, USA). The difference between means was determined by Student's *t*-test in normally distributed data; the non-parametric (Mann–Whitney) test was used for skewed data. Proportions were compared by χ^2 tests. Statistical significance was taken at the 5 per cent level.

Results

Of the 151 infants and children studied, 145 (96 per cent) were breastfed. The mean ± standard deviation (SD) weight for age (Z score) was −1.6 ± 1.2. The average family size was 5.5 and 75 per cent of the families lived in a one-room house. Sixty-eight (45 per cent) children were *H. pylori* positive. *H. pylori* colonization was detected even at the age of 1 month. Ten (50 per cent) of the 1-month-old infants were positive for *H. pylori* and 30 (52 per cent) under 6 months were positive. Diarrhoeal morbidity during the initial 1 month and the entire 6-month follow-up period was then compared between *H. pylori*-positive and *H. pylori*-negative children. Age, nutritional status, family size, and income were comparable between these two groups (Table 1). However, the proportion of male children was greater in the *H. pylori*-negative group (69.8 per cent in the negative group and 48.5 per cent in the positive group were male). The girls had a 70 per cent higher risk of *H. pylori* infection ($\chi^2 = 7.1, p = 0.008$). Six (8.8 per cent) children in the positive group discontinued breast milk feeding whereas all children in the negative group continued taking breast milk. Only three (4.4 per cent) children in the positive group and four (4.8 per cent) in the negative group suffered from diarrhoea during the initial 1-month period following the breath test (Table 2). The diarrhoeal morbidity during the 6-month follow-up

TABLE 2
Incidence of diarrhoea during the month following detection of H. pylori

Days with diarrhoea	H. pylori-positive (n = 68)	H. pylori-negative (n = 83)
0, n (%)	65 (95.6)	79 (95.2)
2, n (%)	0	2 (2.4)
3, n (%)	3 (4.4)	2 (2.4)

TABLE 3
Diarrhoea episodes (per child) during 6-months follow-up

Diarrhoea episode	H. pylori-positive (n = 68)	H. pylori-negative (n = 83)
Infants aged 1–11 months		
0, n (%)	22 (51.2)	21 (41.2)
1, n (%)	13 (30.2)	14 (27.4)
≥2, n (%)	8 (18.6)	16 (31.4)
Median (range)	1.0 (1.0–3.0)	2.0 (1.0–5.0)[a]
Children aged 12–23 months		
0, n (%)	14 (56.0)	19 (59.4)
1, n (%)	5 (20.0)	7 (21.8)
≥2, n (%)	6 (24.0)	6 (18.8)
Median (range)	2.0 (1.0–4.0)	1.0 (1.0–5.0)[b]
All children		
0, n (%)	36 (52.9)	40 (48.2)
1, n (%)	18 (26.5)	21 (25.3)
≥2, n (%)	14 (20.6)	22 (26.5)
Median (range)	1.0 (1.0–4.0)	2.0 (1.0–5.0)[c]

[a] $p = 0.12$; [b] $p = 0.97$; [c] $p = 0.19$ (Mann–Whitney test).

period is shown in Tables 3 and 4. The number of diarrhoea episodes were no different between the *H. pylori*-positive and -negative groups. The median durations (number of days) of diarrhoea were 4.0 (range 1.0–19.0 days) and 5.0 (range 1.0–19.0 days) in the *H. pylori*-positive and -negative groups respectively ($p = 0.29$, Mann–Whitney test). In age-stratified analysis the diarrhoeal morbidity also did not differ between the two groups among infants (<12 months) and children (≥12 months). The mean ± SD weight gain (g/day) in

TABLE 4
Duration of diarrhoea during 6-months follow-up

Cumulative days with diarrhoea	H. pylori-positive (n = 68)	H. pylori-negative (n = 83)
Infants aged 1–11 months		
0, n (%)	22 (51.2)	21 (41.2)
1–4, n (%)	17 (39.5)	14 (27.4)
5–9, n (%)	2 (4.7)	8 (15.7)
≥10, n (%)	2 (4.7)	8 (15.7)
Median (range)	4.0 (1.0–19.0)	5.0 (1.0–19.0)[a]
Children aged 12–23 months		
0, n (%)	14 (56.0)	19 (59.4)
1–4, n (%)	5 (20.0)	7 (21.8)
5–9, n (%)	3 (12.0)	3 (9.4)
≥10, n (%)	3 (12.0)	3 (9.4)
Median (range)	5.0 (1.0–16.0)	4.0 (1.0–16.0)[b]
All children		
0, n (%)	36 (52.9)	40 (48.2)
1–4, n (%)	22 (32.3)	21 (25.2)
5–9, n (%)	5 (7.4)	11 (13.3)
≥10, n (%)	5 (7.4)	11 (13.3)
Mean ± SD	4.0 (1.0–19.0)	5.0 (1.0–19.0)[c]

[a] $p = 0.10$; [b] $p = 0.81$; [c] $p = 0.29$ (Mann–Whitney test).

the two groups over the 6-month follow-up period was similar (7.4 ± 5.7 vs. 7.0 ± 4.7; $p = 0.69$).

Discussion

The present study demonstrated a high prevalence (45 per cent) of H. pylori colonization in infants and children, but this was not associated with any increase in morbidity from diarrhoea. This high prevalence of H. pylori infection could be due to poor living conditions and poor hygiene, as shown in other studies.[1,6] All of the potential risk factors for acquisition of H. pylori infection such as overcrowding, use of a non-sanitary latrine, and use of contaminated water for bathing and cooking were present.

Asymptomatic H. pylori colonization in children in developing countries has been reported previously.[13] The source of H. pylori infection in very young breastfed infants could be their parents and poor environmental sanitation.[14–16] However, an earlier report from the same community examined the intrafamilial clustering of H. pylori infection, which showed an almost equal infection rate among the family contacts of the infected and non-infected children, suggesting that environmental factors may be more important than intrafamilial transfer.[17]

It is well documented that H. pylori infection in children and adults causes chronic upper gastrointestinal problems such as gastritis, gastric ulcer, duodenitis, and abdominal pain.[18,19] Although an association between H. pylori infection and chronic diarrhoea with malnutrition has been reported in the Gambia,[5] we did not find any association between H. pylori colonization and diarrhoea in apparently healthy infants and children in this prospective study; we found that H. pylori colonization in infants and young children is largely asymptomatic. However, we do not know whether they had gastritis or duodenitis, which usually accompany H. pylori colonization.[18,19]

Also, chronic diarrhoea and malnutrition have been associated with H. pylori infection.[5] In the present study, however, nutritional status was very similar in both H. pylori-positive and -negative children. After adjusting for nutritional status and age of the child in stratified analysis, there was no difference in diarrhoeal incidence and duration for H. pylori infected and non-infected children (data not shown). The living conditions, water supply, and latrine type were the same for the entire population. After controlling for these confounders we found no association of H. pylori infection with morbidity due to diarrhoea.

In the present study the urea breath test was done at the beginning of the study to detect H. pylori colonization and was not repeated. With such a common infection it would seem likely that many of the uninfected children would become colonized during the 6-month follow-up period, which would narrow the difference between diarrhoea occurrences in the H. pylori-positive and -negative groups. It is also possible that in some of the children, H. pylori infection was cleared up during

the 6-month follow-up period.[20] However, the association of H. pylori positivity and diarrhoeal morbidity during the first 1-month period immediately following the test is less likely to be confounded by subsequent colonization by H. pylori. In H. pylori-positive children only 4.4 per cent had mild diarrhoea (4.8 per cent in H. pylori-negative children) during the initial 1-month period. Finally, a high proportion (45 per cent) of H. pylori-positive children was free from diarrhoea when the diagnosis was made, suggesting that the H. pylori colonization was not associated with diarrhoea.

In conclusion, H. pylori colonization occurs in early infancy which is not associated with diarrhoea.

References

1. Sack GH, Pierce NF, Hennessy KN, Mitra RC, Sack RB, Mazumder DGN. Gastric acidity in cholera and non-cholera diarrhoea. Bull WHO 1972; 47: 31–6.
2. Giannella RA, Broitman SA, Zamcheck N. Influence of gastric acidity on bacterial and parasitic enteric infections. A perspective. [Review.] Ann Intern Med 1973; 78: 271–6.
3. Morris A, Nicholson G. Ingestion of Campylobacter pyloridis causes gastritis and raised fasting gastric pH. Am J Gastroenterol 1987; 82: 192–9.
4. Graham DY, Alpert LC, Smith JL, Yoshimura HH. Iatrogenic Campylobacter pylori infection is a cause of epidemic achlorhydria. Am J Gastroenterol 1988; 83: 974–80.
5. Sullivan PB, Thomas JE, Wight DG, et al. Helicobacter pylori in Gambian children with chronic diarrhoea and malnutrition. Arch Dis Child 1990; 65: 189–91.
6. Klein PD, Graham DY, Gaillour A, Opekun AR, O'Brian Smith E. Water source as risk factor for Helicobacter pylori infection in Peruvian children. Gastrointestinal Physiology Working Group. Lancet 1991; 337: 1503–6.
7. Perez-Perez GI, Taylor DN, Bodhidatta L, et al. Sero-prevalence of Helicobacter pylori infections in Thailand. J Infect Dis 1990; 161: 1237–41.
8. Graham DY, Adam E, Reddy GT, et al. Seroepidemiology of Helicobacter pylori infection in India: comparison of developing and developed countries. Digest Dis Sc 1991; 36: 1084–8.
9. Mahalanabis D, Rahman MM, Sarker SA, et al. Helicobacter pylori infection in infants and children in a poor community of Bangladesh: prevalence, socioeconomic and nutrition aspects. Int J Epidemiol 1996; 25: 894–8.
10. Dill S, Payne-James JJ, Misiewicz JJ, et al. Evaluation of ^{13}C-urea breath test in the detection of Helicobacter pylori and in monitoring the effect of tripotassium dicitrato-bismuthate in non-ulcer dyspepsia. Gut 1990; 31: 1237–41.
11. Logan RP, Polson RJ, Misiewicz JJ, et al. Simplified single sample 13-carbon urea breath test for Helicobacter pylori: comparison with histology, culture, and ELISA serology. Gut 1991;32:1461–4.
12. Vandenplas Y, Blecker U, Devreker T, et al. Contribution of ^{13}C-urea breath test to the detection of Helicobacter pylori gastritis in children. Pediatrics 1992; 90: 608–11.
13. Blecker U, Hauser B, Lanciers S, Peeters S, Suys B, Vandenplas Y. The prevalence of Helicobacter pylori-positive serology in asymptomatic children. J Pediatr Gastroenterol Nutr 1993; 16: 252–6.
14. De-Giacomo C, Lisato L, Negrini R, Licardi G, Maggiore G. Serum immune response to Helicobacter pylori in

children: epidemiologic and clinical applications. J Pediatr 1991; 119: 205–10.

15. Mitchell JD, Mitchell HM, Tobias V. Acute *Helicobacter pylori* infection in an infant associated with gastric ulceration and serological evidence of intra-familial transmission. Am J Gastroenterol 1992; 87: 382–6.

16. Mitchell HM, Bohane T, Hawkes RA, Lee A. *Helicobacter pylori* infection within families. Int J Med Microbiol Virol Parasitol Infect Dis 1993; 280: 128–36.

17. Sarker SA, Rahman MM, Mahalanabis D, *et al*. Prevalence of *Helicobacter pylori* infection in infants and family contacts in poor Bangladesh community. Digest Dis Sc 1995; 40: 2669–72.

18. Blaser MJ, Perez-Perez GI, Lindenbaum J, *et al*. Association of infection due to *Helicobacter pylori* with specific upper gastrointestinal pathology. Rev Infect Dis 1991; 13: S704–8.

19. Prieto G, Polanco I, Larrauri J, Rota L, Lama R, Carrasco S. *Helicobacter pylori* infection in children: clinical, endoscopic, histologic correlations. J Pediatr Gastroenterol Nutr 1992; 14: 420–5.

20. Klein PD, Gilman RH, Leon-Barua R, Diaz F, Smith EO, Graham DY. The epidemiology of *Helicobacter pylori* in Peruvian children between 6 and 30 months of age. Am J Gastroenterol 1994; 89: 2196–200.