CONCISE COMMUNICATION

Association between *Helicobacter pylori* Infection and Increased Risk of Typhoid Fever

Maharaj K. Bhan,[1] Rajiv Bahl,[1] Sunil Sazawal,[3]
Anju Sinha,[1] Ramesh Kumar,[1] Dilip Mahalanabis,[2]
and John D. Clemens[4]

[1]*Advanced Center for Diarrheal Disease Research, Department of Pediatrics, All India Institute of Medical Sciences, New Delhi, and* [2]*Society for Applied Studies, Kolkata, India;* [3]*Department of International Health, Johns Hopkins University, Baltimore, Maryland;* [4]*International Vaccine Institute, Seoul, Korea*

Helicobacter pylori infection has been reported to increase the risk of cholera. This nested case-control study was conducted to determine whether *H. pylori* infection is associated with occurrence of typhoid fever. Eighty-three case subjects of culture-proven typhoid fever were identified through a 1-year surveillance of subjects aged 0–40 years in an urban slum. Two age- and sex-matched neighborhood control subjects were concurrently selected for each case subject. Serum anti–*H. pylori* immunoglobulin G antibodies were measured in case and neighborhood control subjects. For determining other risk factors, 2 additional community control subjects per case were selected. There was a significant association between the presence of serum anti–*H. pylori* immunoglobulin G antibodies and typhoid fever (adjusted odds ratio, 2.03; 95% confidence interval, 1.02–4.01). Illiteracy, being part of a nuclear family, nonuse of soap, and consumption of ice cream were also associated with a significantly greater risk of typhoid fever. This study provides the first empiric evidence that *H. pylori* infection is associated with an increased risk of typhoid fever.

Helicobater pylori infection was associated with epidemic *Vibrio cholerae* O1 infection in Peru [1]. A Bangladesh-based article reported an increased risk of endemic cholera of life-threatening severity in that country [2]. This effect is probably mediated through hypochlorhydria, which is a common finding in developing countries and an important risk factor for cholera. Acute *H. pylori* infection is known to cause hypochlorhydria that can persist for >8 months [3]. Chronic *H. pylori* infection can cause hypochlorhydria by causing atrophic gastritis.

The gastric-acid barrier is an important determinant of the severity of salmonella and shigella infection [4]. We hypothesized that *H. pylori* infection would increase susceptibility to *Salmonella typhi* infection, as a result of the induced hypochlor-

hydria. Surveillance for typhoid fever was conducted in an urban slum community in south Delhi from November 1995 to October 1996 to determine the incidence of typhoid fever, and these findings were published elsewhere [5]. We performed a case-control study nested within the above longitudinal study to determine whether *H. pylori* infection is a risk factor for typhoid fever. In addition, potential associations of socioeconomic status, water source, sanitation, hygiene, and dietary factors with typhoid fever were examined.

Subjects and Methods

Surveillance for identification of typhoid case subjects. In 1995, a computerized census of the population of the study area showed a total of 19,585 residents living in 4361 dwellings. All residents were assigned a unique identification number, and this gave us the sample population for the study. The population was divided into clusters of 70 households, with a mean of 4.49 persons/household, and 26 such clusters were randomly selected by computer for active surveillance [5]. The residents in the remaining household clusters were kept under passive surveillance by health facilities and neighboring practitioners for detection of cases of fever.

Subjects who reported fever were identified though active and passive surveillance. Blood specimens for culture were obtained from those ⩽5 years old if they had a body temperature ⩾38°C, irrespective of the duration of fever. Subjects >5 years old had to have had continuous fever for the previous 3 days to have a blood culture performed. A passive surveillance system was established that included all the medical facilities serving the community at which blood specimens could be drawn.

Table 1. Risk factors for typhoid fever other than *Helicobacter pylori* among adults and children from an urban slum in Delhi

Possible risk factor	No. (%) of case subjects ($n = 83$)	No. (%) of control subjects ($n = 324$)	Matched OR (95% CI)	
			Crude	Adjusted[a]
Ownership of dwelling	76 (92)	310 (96)	0.5 (0.2–1.29)	0.45 (0.15–1.31)
Nuclear family	67 (81)	206 (64)	2.33 (1.29–4.21)[b]	2.55 (1.36–4.78)[b]
No family member literate	6 (7)	6 (2)	3.91 (1.26–12.1)[b]	3.44 (1.04–11.4)[b]
Typhoid case in the family in previous 12 months	16 (19)	49 (15)	1.37 (0.72–2.61)	1.5 (0.76–2.96)
Dirty container for storing drinking water	79 (95)	290 (90)	2.43 (0.82–7.15)	1.99 (0.6–6.65)
Nonuse of soap for washing hands	41 (49)	125 (39)	1.69 (1.0–2.86)[b]	1.82 (1.04–3.21)[b]
Water or drinks outside home in the previous 3 weeks	47 (56)	157 (48)	1.4 (0.8–2.3)	1.4 (0.77–2.57)
Lunch or dinner outside home in the previous 3 weeks	15 (18)	38 (12)	1.8 (0.9–3.58)	1.27 (0.6–2.68)
Consumption of ice cream in the previous 3 weeks	34 (41)	78 (24)	2.54 (1.44–4.48)[b]	2.5 (1.35–4.61)[b]

NOTE. CI, confidence interval; OR, odds ratio.
[a] Conditional logistic regression, with all the variables shown in the table in the model as covariates.
[b] $P < .05$.

Case-control study to determine the association between H. pylori *and typhoid fever.* A total of 97 case subjects with culture-positive typhoid fever were identified. Of these, 14 were excluded from this case-control study because they were relapsed case subjects ($n = 3$), out-migrated immediately after diagnosis ($n = 5$), or a blood specimen for *H. pylori* serologic testing could not be obtained ($n = 6$).

For each of the remaining 83 case subjects (within 70 households), 2 neighborhood sex- and age-matched (± 2 years) control subjects from the same residence block as the case subject were selected within a week of onset of fever. For this purpose, a list of eligible control subjects from the 10 houses preceding and 10 houses following the residence of the case was generated from the computerized database. Those who had previously been diagnosed as having had typhoid fever were removed, and the remainder were arranged in a random order. These households were visited in this predetermined sequence until 2 control subjects were interviewed and a blood sample from them was obtained. None of the control subjects with fever on the day of selection had a blood culture positive for *S. typhi.*

A fingerprick blood sample from case subjects and control subjects was collected on 3-mm filter paper for *H. pylori* IgG detection (Whatman). The paper was air dried, stored in ziplocked plastic bags, and frozen at $-20°C$ until analysis. Presence of *H. pylori* IgG antibodies was tested by a commercially available ELISA (Pylori Stat Test Kit; BioWhittaker) for serum. Five 5-mm filter paper discs punched out from the blood spot that had ~10 μL blood each were soaked in 300 μL buffer, and the supernatant was used as the test serum. This optimum 1:6 dilution of blood was determined prior to testing specimens from study subjects, as it achieved results similar to the manufacturer recommended 1:20 dilution of serum; the values obtained by 1:10 dilution of blood were lower than those with 1:20 dilution of serum, even though both contained similar amounts of serum.

Case and control subjects were queried by the same trained field workers within a week of their selection for personal and family characteristics, socioeconomic indicators, and the previous 3 weeks' food and drink that were consumed by case subjects (before onset of fever) and matched control subjects. The interviewer also made direct observations on water handling, garbage disposal, and availability of soap for hand-washing.

Case-control study to determine other risk factors for typhoid fever. The case subjects and matched neighborhood control subjects for

this analysis were the same as those selected for testing the hypothesis related to *H. pylori.* Furthermore, 2 additional control subjects per case subject, not living in the case subject's community, were randomly selected from the computerized database to avoid overmatching for risk factors like water and sanitation that were expected to be common to persons living in the immediate vicinity. The community control subjects were interviewed in the same manner as the case subjects and neighborhood control subjects, but blood samples for serologic testing for *H. pylori* were not obtained.

Data analysis. STATA 6 software (Stata Corp) was used for statistical analysis. Matched odds ratios (ORs) and 95% confidence intervals (CIs) were estimated. Conditional logistic regression models were used to adjust the ORs for different risk factors for potential confounders, such as age, family size, ownership of dwelling, source of drinking water, open garbage within the house, and food consumed outside home in the 3 weeks preceding the date of selection as case or control. ORs were used to calculate the etiologic fraction of disease (i.e., the fraction of typhoid fever case subjects that might be attributed to *H. pylori* infection using standard methods). The ORs presented would approximate relative risks because of the rarity of typhoid fever in the community (incidence, 9.8/1000).

Results

Serum anti–*H. pylori* IgG antibodies were detected in 64% of case subjects (53 of 83) and 50% of neighborhood control subjects (83 of 166) (crude matched OR, 1.81; 95% CI, 1.03–3.17). This association remained significant after adjustment for potential confounders in a conditional logistic regression model (adjusted OR, 2.03; 95% CI 1.03–4.01). The etiologic fraction of typhoid due to *H. pylori* was ~34%. The potential confounders that were adjusted for included age, ownership of dwelling, type of family, literacy, condition of storage of drinking water, use of soap for hand-washing, and consumption of food, water, or ice cream outside of the home in the previous 3 weeks.

Table 1 shows the association between typhoid fever and other possible risk factors. Because the associations were similar when neighborhood or community control subjects were compared with case subjects (data not shown), we have presented

a combined analysis with 4 control subjects (2 neighborhood and 2 community control subjects) per case subject. Illiteracy (adjusted OR, 3.44; 95% CI, 1.04–11.4) and living in a nuclear family (adjusted OR, 2.29; 95% CI, 1.36–4.78) were significantly associated with a greater risk of typhoid fever. Not using soap for washing hands was another important risk factor for typhoid (adjusted OR, 1.82; 95% CI, 1.04–3.21). Among foods consumed during the 3 weeks prior to onset of fever, only ice cream consumption was associated with typhoid fever (adjusted OR, 2.5; 95% CI, 1.35–4.61).

Discussion

This is the first study to indicate that *H. pylori* infection is associated with an increased risk of typhoid fever. Illiteracy, non-use of soap for washing hands, and consumption of ice cream were other significant risk factors for typhoid fever in this urban slum population. The results were consistent for case-control comparisons derived from active and passive surveillance areas and for different age categories (data not shown).

Whether the observed association between *H. pylori* infection and typhoid fever is free of bias and is causal merits careful consideration. The strength of association was moderate (OR, ~2). Because the study was nested within a cohort and the control selection was performed concurrently, the selection bias is likely to be small. Presence of serum IgG antibodies suggests active or previous infection with *H. pylori,* as antibodies may persist beyond clearance of infection, thus reducing the validity of the assay in detecting active infection [6]. However, because the serum samples from case and control subjects were tested with identical methods, any misclassification of exposure is likely to be non-differential and should have acted to diminish, rather than augment, the observed association. Serum IgG antibodies to *H. pylori* develop 1–3 months after an initial infection. We measured *H. pylori* antibodies within a week of selection of case subjects (i.e., onset of fever) or of control subjects. This indicates that the *H. pylori* infection had preceded occurrence of typhoid fever. Lastly, although the association between *H. pylori* infection and typhoid fever remained significant even after adjustment for many socioeconomic and life-style factors, there could be genetic and other environmental confounders that were not measured and therefore were not adjusted.

The gastric-acid barrier has been shown elsewhere to be an important protective mechanism against salmonella infections [4]. Acute and chronic *H. pylori* infections are both known to cause hypochlorhydria and, thus, compromise the gastric-acid barrier [3], this may be the mechanism by which such infections increase the risk of typhoid fever. A significant association between *H. pylori* infection and depressed gastric-acid output in The Gambia was found in a setting similar to the one in which the present study was performed [7]. This is probably the mechanism through which the increased risk of typhoid fever is mediated. Furthermore, there is a theoretical, but as yet unsubstantiated, possibility

that the chronic *H. pylori* infection required to produce an IgG response may alter the mucosal immune response in a way that increases the risk of developing typhoid fever following *S. typhi* ingestion.

In 2 previous studies, the presence of IgG antibodies to *H. pylori* was associated with increased risk of cholera [1, 2]. Whereas another 2 studies reported no increase in diarrhea after colonization with *H. pylori,* as assessed by breath test [8, 9], a recent study showed that presence of IgG antibodies to *H. pylori* was followed by an increase in diarrheal disease among Peruvian children [10]. The present study found an increased risk of typhoid fever in those who had antibodies to *H. pylori.* It may be that established infection, reflected by gastritis and detectable immune response, rather than mere colonization with *H. pylori,* is required for the development of hypochlorhydria and increased risk of enteric infections.

Previous studies from Pakistan, Indonesia, and Chile have reported that eating ice cream, flavored ices, or food bought from outside the home, not washing hands before eating, non-municipal water supply or drinking water at work site, and open sewers are risk factors for endemic typhoid [11–13]. The present study also highlighted eating ice cream and not washing hands with soap as important risk factors of typhoid. We did not find water source and sanitation to be important risk factors, although there was indeed little variability in these factors in the community.

The findings of this study have particularly important implications for children and adolescents. *H. pylori* infections occur widely among children in developing countries [14, 15]. The significance of this childhood infection and the priority to be accorded to its prevention would depend on its association with risk of gastro-duodenal disease in adult life and its other effects in childhood. Studies elsewhere have shown that *H. pylori* infection is associated with increased risk of cholera and diarrhea [1, 2, 10]. Our results show an association of *H. pylori* infection with typhoid fever and therefore provide additional evidence that will contribute to addressing the issue of whether it is important to prevent *H. pylori* infection in developing country children.

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