Age at acquisition of *Helicobacter pylori* infection: a follow-up study from infancy to adulthood

Hoda M Malaty, Abdalla El-Kasabany, David Y Graham, Charles C Miller, Sidd G Reddy, Sathanur R Srinivasan, Yoshio Yamaoka, Gerald S Berenson

Summary

Background *Helicobacter pylori* infection is common worldwide, but the time of acquisition is unclear. We investigated this issue in a cohort of children selected retrospectively from a population followed up for 21 years.

Methods We monitored 224 children (99 black, 125 white; 110 male, 114 female) from 1975–76 (ages 1–3 years) to 1995–96. *H pylori* status was assessed by presence of serum IgG antibodies.

Findings 18 (8·0%) children at age 1–3 years had *H pylori* antibodies (13% black *vs* 4% white children, p=0·008). By age 18–23 years, the prevalence of the infection was 24·5% (43% black *vs* 8% white participants, p<0·0001). Of the 206 children not infected at baseline, 40 (19%) became infected by age 21–23. The crude incidence rate per year was 1·4% for the whole cohort, ranging from 2·1% at 4–5 years and 1·5% at age 7–9 years to 0·3% at 21–23 years. The seroconversion rate was higher among black than among white children (relative risk 3·3, 95% CI 1·8–6·2, p=0·001). The median age for seroconversion was 7·5 years for both races. Nine of the 58 seropositive children cleared the infection during follow-up. The rate of seroreversion per year was 1·1%; it was highest among children at age 4–5 years (2·2% *vs* 0·2% at ages 18–19).

Interpretation Most newly acquired *H pylori* infections happened before age 10 years. Treatment and preventive strategies should be aimed at children in this age-group.

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Department of Medicine (H M Malaty MD, Prof D Y Graham MD, S G Reddy MPH, Y Yamaoka MD) **and Department of Molecular Virology and Microbiology** (D Y Graham)**, Veterans Affairs Medical Center and Baylor College of Medicine, Houston, TX; Tulane Center for Cardiovascular Health, Tulane School of Public Health, New Orleans, LA** (A El-Kasabany MD, Prof S R Srinivasan PhD, Prof G S Berenson MD)**; and Department of Surgery, University of Texas Medical School, Houston, TX, USA** (C C Miller PhD)

Corespondence to: Dr Hoda M Malaty, Veterans Affair Medical Center (111D), 2002 Holcombe Boulevard, Houston, TX 77030, USA (e-mail: hmalaty@bcm.tmc.edu)

Introduction

Gastritis, gastric ulcer, duodenal ulcer, gastric carcinoma, and primary gastric B-cell lymphoma are all aetiologically associated with *Helicobacter pylori* infection.[1–3] Childhood is the time of high risk for *H pylori* acquisition,[4–6] but the peak age of acquisition is still unclear and could differ among populations. An understanding of the epidemiology of *H pylori* infection in childhood is required to elucidate fully its natural history.

H pylori infection of the gastric mucosa leads to local and systemic immune responses. Measurement of serum IgG antibodies by ELISA is a reliable, inexpensive, and non-invasive way to detect the infection and is useful in epidemiological studies.[7,8] The prevalence of *H pylori* in a community depends on the rate of acquisition and the rate of loss of the infection. The seroconversion rate is an indicator of the rate of acquisition of the infection, and the seroreversion rate reflects loss of infection. Seroconversion and seroreversion rates are important factors for definition of the best time for therapeutic or immunising interventions.

The epidemiology and risk factors associated with *H pylori* infection have been intensively investigated.[9–12] Studies from the USA have reported ethnic differences in the age-specific prevalence of *H pylori* infection in children, with black and hispanic children having the highest rates of acquisition.[13,14] Most of the epidemiological studies have been cross-sectional, and thus could not gauge the rates of acquisition and loss of the infection.

In this study, we examined the age at which the rate of acquisition of the infection was highest and the effects of race and sex on the acquisition of *H pylori* infection. We studied a representative sample of a longitudinal cohort of infants monitored up to age 21 years as part of the Bogalusa heart study, which provided a unique opportunity to examine *H pylori* status from infancy to adulthood in a well-defined biracial community.

Methods

Study population

The Bogalusa heart study is being done in the community of Bogalusa, LA, USA. This epidemiological study aimed to examine risk factors for cardiovascular disease from birth through to adulthood. The population is well defined and biracial (35% black, 65% white), with a homogeneous lower middle socioeconomic class. A mixed epidemiological design has included cross-sectional surveys, 3–5 years apart, and longitudinal surveys to obtain detailed observations on more than 12 000 people from birth to adulthood among the general population of about 22 000 people. The first detailed cross-sectional screening began in 1973–74 on school-age children who have subsequently been studied both cross-sectionally and longitudinally.

Newborn and preschool-age cohorts were added between 1974 and June, 1975, when clinical, laboratory, and demographic data were collected on 440 babies born to Bogalusa residents. The infants were examined at ages

6 months, and 1, 2, 3, and 4 years, and then in school surveys at ages 5, 7, 10, and 13 years, and in adulthood. In this cohort, 80% were examined at age 6 months and 57% at 7 years.

At each examination, children underwent a medical history interview, physical examination, blood sampling, and a series of laboratory tests. Further details of the procedures, sample, and design of the study have been reported previously.[15]

We studied a sample of a longitudinal cohort monitored as newborn infants in 1975–76 to young adults in 1996 with repeated blood samples and questionnaires within the framework of Bogalusa heart study. Participants were identified by the child's identification number and the year of data collection through a computer program that generated a longitudinal database for the cohort. We identified 247 newborn children, of whom 226 met the criteria of having serum samples available in 1975–76 at ages 1, 2, or 3 years, and at least two consecutive blood samples in following years. These children were re-examined in 1976–77, 1978–79, 1981–82, 1987–88, 1992–93, and 1995–96.

Study protocol

Serum samples were identified by year of collection. The numbers of serum samples that were available for each examined interval were: 132 samples in 1976–77, 102 in 1978–79, 127 in 1981–82, 84 in 1987–88, 98 in 1992–93, and 45 in 1995–96.

The samples had been stored at a serum bank at –70°C. Information on potential *H pylori* risk factors including age, race, and sex, was obtained for each child. *H pylori* eradication therapy was not used during the study.

Concentrations of IgG antibody to the high-molecular-weight cell-associated proteins of *H pylori* were measured by ELISA (HM-CAP, EPI, Westbury, NY, USA). The test was scored as positive when the optical density was more than 2·2. The assay has sensitivity of more than 98% and specificity of more than 95% for adults;[7] it has also been validated in children (sensitivity 100%, specificity 96%).[8]

Immunoblot assays were done with HelicoBlot 2.1 (HB2.1; Genelabs Diagnostics, Singapore) according to the manufacturer's recommendations. This preparation contains several antigens including 116 K (CagA), 89 K (VacA), 37 K, 35 K, 39 K (UreA), and 19·5 K. A serum sample was considered positive for *H pylori* if it reacted with any one band at 89 K, 37 K, or 35 K, or both bands at 30 K and 19·5 K. A serum sample was classified as CagA-positive if it reacted with 116 K antigens and also passed the criteria for *H pylori* infection. Serum samples that had no reactivity to any bands, or reactivity that did not meet the criteria for positivity, were reported as negative. This assay showed sensitivity and specificity above 95% for detection of *H pylori* infection when the status was based on the combination of serum ELISA, urea breath test, and histology results (unpublished). The sensitivity and specificity for CagA status in HelicoBlot 2.1 were 98% and 90% when CagA status was based on PCR and Southern hybridisation, respectively.

Acquisition of *H pylori* infection was defined as *H pylori* IgG seroconversion. We reassayed all serum samples from children with differences from baseline antibody results. Furthermore, the western blot assay was done on masked serum from 30 of the seroconverted cases to ensure the accuracy of the date when the new antibodies developed. After obtaining the immunoblot results, we used the results of both tests to show the comparison of the seroconversion pattern within each seroconverter during the follow-up years. Two seroconverted children had discrepant results between the ELISA and the western blot assay and were excluded from the analysis to avoid any potential bias in the interpretation of the results. The date of acquisition of *H pylori* infection was defined as the date of the first positive result for *H pylori* IgG antibodies.

Statistical analysis

The study had a historical cohort design. Each participant was followed up longitudinally from the date that blood samples were drawn. Participants who were positive for *H pylori* at baseline were counted as seropositive but not as seroconverters. The frequency of *H pylori* seroconversion was calculated as the number of new cases in an assessment period divided by the number of cohort members still at risk. This frequency was annualised by dividing the risk period results by the number of years in the period. The cohort was grouped according to date of birth, when the blood sample was taken, and according to age, race, and sex. Differences between race and sex groups were assessed on cumulative incidence data for the whole period by standard relative-risk statistics. Seroreversion was calculated similarly, with the number of confirmed cases reverting during each period as the incidence measure. Only confirmed cases of reversion with negative results for the remainder of the observation period, which had to include at least two negative samples, were counted as seroreversions.

Predictor variables available for hypothesis testing were age, sex, and race. We calculated two-by-two contingency-table statistics to examine each of the sex and race relations with cumulative incidence over the entire period. Contingency-table relative risks with test-based CI were calculated for the two-by-two tables (STAT, version 6). We did not do statistical tests on the age-group prevalence described above because of the difficulty of missed visits and the effect of these on the denominator. Our primary endpoint was age at acquisition, so we considered the first seroconversion to be a failure and we used life-table statistics to calculate the hazard function for acquisition of *H pylori* over the whole measured age range. The hazard function is the instantaneous probability of acquisition during a particular period, with actuarial denominators. We included cases positive at baseline in the failure-time analyses, because all of them must have converted before the age of 2 years, and we considered loss of that information to be unacceptable when attempting to model risk factor effects over time. We calculated multivariate Cox's proportional-hazards

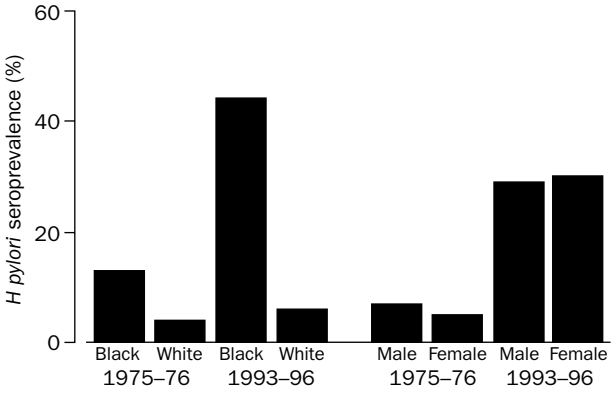

Figure 1: **Effect of race and sex on seroprevalence of *H pylori* infection in children in 1975–76 and in 1993–96 as young adults**

regression statistics to estimate the effects of race and sex on incidence. Failure-time statistics provide adjustments for incomplete follow-up in the cohort and for the correlation inherent in repeated measures.

Role of the funding source

The sponsors of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

Results

The cohort consisted of 224 children, of whom 99 (44%) were black and 125 were white; 110 (49%) were male and 114 (51%) female. The frequency of seropositivity for *H pylori* increased from 8·0% (18/224) at ages 1–3 to 24·5% (35/143) at ages 18–23 (p=0·002). The serum samples from the 18 young children who were seropositive at study entry were also tested by the immunoblot assay and there was 100% concordance.

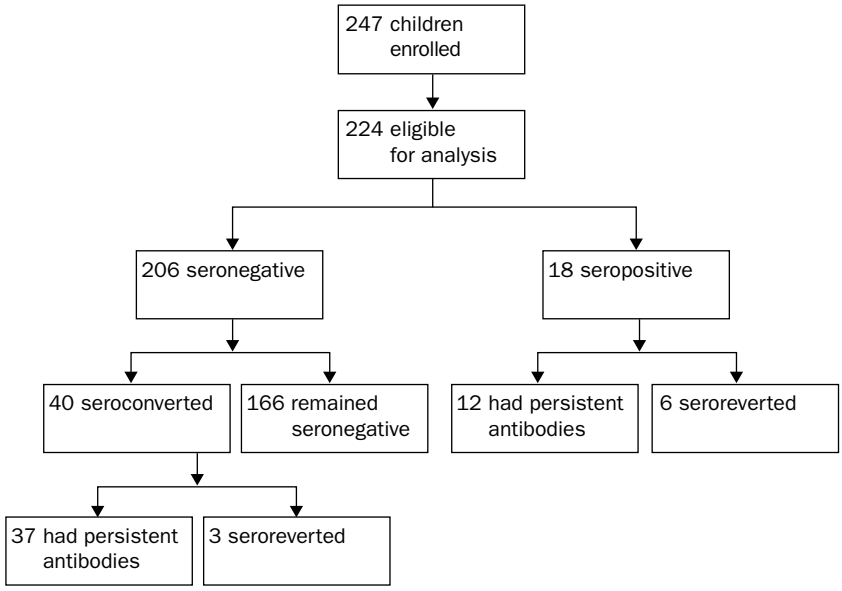

Figure 3: **Pattern of seroepidemiology**

The frequency of seropositivity differed significantly between black and white children (p=0·008) and the difference remained throughout follow-up. No significant difference was observed between male and female participants during childhood or during early adulthood (figure 1). In multivariate Cox's analyses, sex was not significant (relative risk male *vs* female 0·841, p=0·52) but race was highly significant (black *vs* white 3·429, p<0·0001).

The mean follow-up for the cohort included in the analyses was 14 years (SD 6) and was identical for black and white children, and for male and female participants. 40 of the 206 children who were seronegative for *H pylori* at study entry seroconverted during follow-up. The crude rate of seroconversion was 1·4% per year for the total cohort, 2·3% per year for black children, and 0·7% per year for white children. Thus, the relative risk of *H pylori* seroconversion was three times higher in black than in white children (3·3 [95% CI 1·8–6·2], p=0·001). In the whole cohort, younger children had the highest seroconversion rates: 2·1% per year for those aged 4–5 years and 1·5% per year for those aged 7–9 years. The age-specific rate of seroconversion decreased to 1% per year at ages 13–15 years and then to the lowest rate, 0·3% per year, in early adulthood (ages 21–23 years; figure 2). The pattern was different for age-specific seroconversion rate within each ethnic group independently, since the median age of seroconversion was the same for black and white children (7·5 years). The crude rate did not differ between female and male children (1·4% *vs* 1·2% per year; relative risk 1·2 [0·7–2·1], p=0·6). Hazard analysis showed an increased risk of acquisition before 5 years of age, a decline in risk from age 5 to 15 years, and a tendency for risk to increase again towards 20 years of age. The standard errors for the older age ranges were large, owing to the decreasing sample size over time; nevertheless, the pattern of the hazard estimates was consistent with the proportion analysis shown in figure 2.

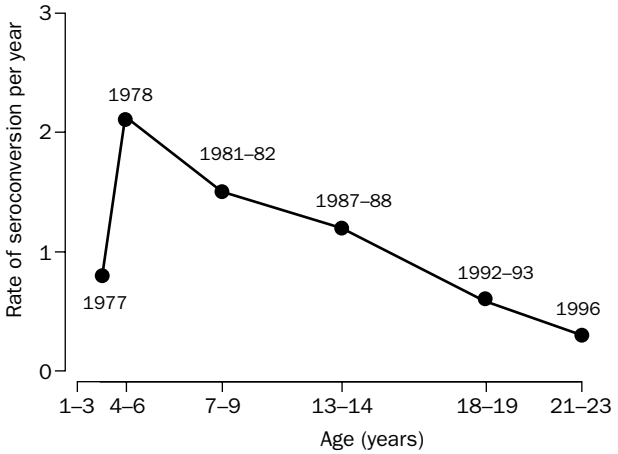

Figure 2: **Age-specific rate per year of seroconverion for *H pylori* among the 206 children who were seronegative in 1975–76 at ages 1–3 years**

58 children were seropositive for *H pylori*; 18 children were seropositive at study entry and 40 seroconverted during follow-up (figure 3). The risk of persistence of *H pylori* antibodies throughout the study was eight times higher among children who acquired the infection during the study than among those who were infected before the study (ie, at ages 1–3 years; relative risk 6·2, 1·3–28·5, p=0·004).

Nine children seroreverted (lost the infection without reacquiring it before the study ended). The crude clearance rate for *H pylori* antibodies was 1·1% per year (1·7% per year among white children and 0·8% per year among black children). The total seroreversion rate was two times higher among white than among black children (1·9 [0·52–9·54], p=0·27). The crude seroreversion rate was 1·3% per year for girls and 0·8% per year for boys (2·8 [0·65–12·5], p=0·14). However, these differences were not significant, possibly owing to the small numbers.

Discussion

Our results indicate that the highest rates of acquisition of the infection were before age 10 years. The frequency of seropositivity differed significantly between black and white children, as seen in cross-sectional studies.[13,14] Socioeconomic status correlates with variation in the prevalence of *H pylori* infection between races and ethnic

groups.[9,10,13,16] In the Bogalusa community, the risk of acquiring a new infection was five times greater among black than white children and the median age at seroconversion was the same for both races (7·5 years). Such differences are important in terms of decisions on therapeutic and management intervention for *H pylori* infection.

Over 84% of the infected children who were initially seropositive or who became seropositive during follow-up remained infected during adolescence and young adulthood. Despite the low rate of seroconversion and low numbers of persistent cases in the study, the total number of *H pylori* cases in the USA is likely to be high. For example, 24% of non-affluent black and white hispanic children born in the USA had active *H pylori* infection as diagnosed by the urea breath test.[13] In that study, 20% of the children were infected by the age of 10 years. The overall rate of clearance of *H pylori* infection was 1·1% per year among the whole cohort. However, there were differences between the two races; black children remained infected (or became reinfected) during the observation period whereas the infection was two times lost among the infected white children. Possible explanations for the higher rate of acquisition and the lower rate of loss of infection among black children include more intense exposure from higher infection rates among family members, differences in household hygiene, or differences in access to or use of health-care facilities.

H pylori infection clusters in families,[17,18] but whether transmission is more often due to sharing a common exposure source or occurs between individuals is unknown. The lower rates of acquisition and the higher rates of loss of *H pylori* infection among white children are consistent with the continuing decrease in the prevalence of the infection in the white population in the USA.[19] Spontaneous elimination of *H pylori* infection (ie, seroreversion) has also been reported in adults and children from more-developed and less-developed countries.[20–24] For example, a study in Sweden[20] followed a cohort of children from age 6 months to 11 years and the results show that spontaneous clearance of the infection was common in young children.[20] Similar results were reported from Finland and Japan.[21,22] In a study in Peru[23] children were examined from age 6 months by the urea breath test. In this trial, infection acquired by very young children did not persist in all instances, which suggests there is a high acquisition rate despite low prevalence among young children at any point in time.[23] The reasons for a higher rate of clearance of *H pylori* infection among white children are also unknown. Loss of infection might be related to the widespread use of antimicrobial drugs for other common infections. *H pylori* eradication therapy had not been discovered during our study.

This study confirms that even within the same community, different cohorts can have different risks of acquiring the infection. Race had an important effect on the acquisition, persistence, and clearance of the infection, whereas sex had no significant effect. *H pylori* infection was acquired at any time between infancy and young adulthood, but early childhood seems to be the important period when most of the infections are acquired. The period in life when the infection is acquired might also affect risk of development of forms of the infection with symptoms such as peptic ulcer, gastric cancer, or primary gastric B-cell lymphoma.[25,26]

The use of data such as these for constructing a retrospective cohort has some shortcomings. First, we have no detailed data on several risk factors that are known to be associated with the acquisition of *H pylori* infection, such as socioeconomic status or structure of the households in which the children lived. Therefore, we were unable to explore fully which risk factors, such as socioeconomic status, genetic predisposition,[27] social and cultural background, and environmental factors, are most important in the acquisition of *H pylori* infection. Second, the small number of seroreverted cases limited the power to examine whether race and sex are risk factors for seroreversion. A final limitation is the suggestion that the sensitivity of serological tests for *H pylori* is lower among young children than among older children and adults.[28] However, the test used has been examined in the US population, and it is a widely accepted tool for studies that have defined the major epidemiological features of *H pylori* infection in childhood. The serological test used for our study has consistently shown a positive predictive value of more than 90% in children,[8,29] and the results were confirmed by immunoblot.

Contributors

H Malaty was responsible for initiating the collaboration, obtaining approval and funding, design, statistical analysis, and writing of the report. A Kasabany was responsible for identifying the information on computer relevant to this study and the infants eligible for it. C Miller was responsible for statistical analysis. S Reddy divided serum samples from the serum bank under the supervision of H Malaty and did the ELISAs. Y Yamaoka did the immunoassays and wrote the relevant part of the report. D Graham contributed expertise on laboratory assays, and helped to write the report. S Srinivasan supervised division of serum samples, quality control, and transfer of samples to Baylor College of Medicine. G Berenson was principal investigator of the Bogulusa Heart Study.

Conflict of interest statement

None declared.

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