MAJOR ARTICLE

Seroepidemiology of *Helicobacter pylori* and Hepatitis A Virus and the Mode of Transmission of Infection: A 9-Year Cohort Study in Rural Japan

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We compared the seroepidemiologic patterns of *Helicobacter pylori* and hepatitis A virus (HAV) infections among participants in 2 independent cross-sectional studies conducted in Japan in 1986 and 1994. Subgroups were monitored with successive blood sampling. *H. pylori* and HAV infection status was defined by results of enzyme-linked immunosorbent assay. In 1986, the prevalence of *H. pylori* infection and HAV infection, respectively, were 80% and 70% among adults and 31% and 5% among children. The prevalence of both infections increased with age. Concordant infections were found in 74.5% of adults ($\kappa = 0.2$) versus 2% of children ($\kappa = 0.05$). During the 9-year study period, the incidence of *H. pylori* infection was 1.1% among adults and 2% among children. The seroprevalence of HAV remained constant. The disparity between the increase in prevalence of *H. pylori* and HAV infection with age is likely associated with improvements in hygienic practices. The discordance between the presence of the infections among younger persons is evidence against a common source and/or vehicle for transmission.

The prevalence of *Helicobacter pylori* infection varies both between and within populations, with the rate of acquisition being generally higher in underdeveloped than in industrialized countries [1–5]. Cross-sectional studies have consistently shown a gradual increase in *H. pylori* seroprevalence with age, which has been interpreted as a birth cohort effect reflecting a decrease in the rate of acquisition in successive generations of children as sanitation improved and standards of living increased [6, 7].

The isolation of *H. pylori* from feces, dental plaque, and saliva [8–10] supports the possibility of fecal-oral transmission of *H. pylori* infection. The change in the seroepidemiologic pattern of *H. pylori* infection was noted to be very reminiscent of the changes that occurred in the last century in the seroprevalence of polio and hepatitis A [11, 12]. Several early epidemiological studies examined the seroprevalence of hepatitis A virus (HAV) and *H. pylori* in the same populations and found that, in general, the seroprevalences were parallel [13, 14]. The likely explanation was that the parallel changes in seroprevalence reflected changes in sanitation and standards of living, but the presence of a common source of infection could not be excluded. A number of cross-sectional studies have compared the seroepidemiologic patterns of *H. pylori* and HAV infection to address whether there was a common infection pathway [15–19]. These studies suffer from some limitations, because their designs only allowed examination of seroprevalence patterns of both infections within a community at a single time point.

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We studied the seroepidemiology of *H. pylori* and HAV in Japanese children and adults from a typical mountain village in the district of Nagano Prefecture, Japan. We report a cohort study and compare the results with the age-specific seroprevalence of both pathogens from 2 independent cross-sectional surveys of the same population conducted in 1986 and 1994.

PATIENTS, MATERIALS, AND METHODS

Study area. The study was performed in a small district of South Kiso town located in central Japan. This district is surrounded by mountains and consists of 19 small communities, with a total population of 1117 inhabitants. The current water supply system was introduced in 1959 and utilizes spring water from 2 locations. Before 1959, the river, wells, and springs were the sources of drinking water. There is a central sewage system.

Study population and serum collection. Serum samples were obtained from adults and children who participated in 2 cross-sectional surveys conducted in the region in 1986 and 1994 within the framework of a study of hepatitis C transmission [20]. There were 649 subjects who participated in the 1986 survey and 143 who participated in the 1994 survey. The populations that participated in the 2 study periods were completely independent. Each individual completed questionnaires. Thereafter, 20 mL of blood was obtained, and the serum was separated. Each serum sample was divided into 3 parts, 1 of which was stored at −80°C until the current study begun.

Of the 649 subjects who participated in the 1986 survey, serum samples from 614 were available to be tested for *H. pylori* and HAV. Of these 614 subjects, 480 (394 adults and 86 children) were monitored from June 1986 through September 1994 with successive blood sampling and questionnaires (figure 1). Blood samples were obtained each year during a 2-day survey conducted in June, July, August, or September. Subjects were eligible for the current longitudinal study if they had at least 2 serum samples in >2 successive years available for testing. *H. pylori* "eradication therapy" was not used during the study period.

Serological testing methods. *H. pylori* infection status was determined by testing for the presence of anti–*H. pylori* IgG antibodies with ELISA using the GAP-IgG Kit (Biomerica). A standard curve was drawn by measuring the absorbance of the reference serum sample included in the kit. The reference serum sample was diluted serially from 1:2 to 1:16 with PBS (pH, 7.2), and the amount of anti–*H. pylori* IgG corresponding to a dilution of 1:8 was expressed as an arbitrary index (AI) of 1.0. The cross-reactivity of the antibody in a patient's serum sample with 2 closely related bacterial strains (4 strains of *Campylobacter jejuni,* 1 strain of *Campylobacter ralidis,* and 1 strain of *Escherichia coli*) was examined as described elsewhere [21, 22]. In brief, serum samples obtained from 10 adults and 10 children (age range, 5–19 years) who were carriers of anti–*H. pylori* IgG were incubated for 30 min at 37°C with the sonicated cell extracts of the bacterial strains, and the level of unabsorbed anti-*H. pylori* IgG was measured. A control test with an authentic *H. pylori* strain (ATCC 43504) was performed in parallel. The ELISA results for this population were validated using a receiver operating characteristic curve to determine the cutoff value (AI, 0.51). The results were compared with those

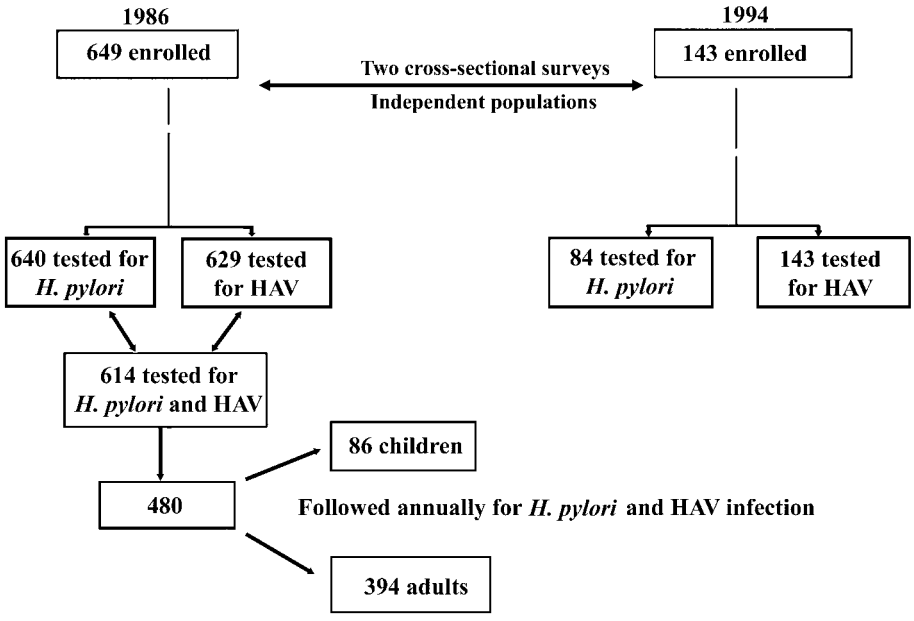

Figure 1. Baseline enrollment and follow-up data of the study population in rural Japan, 1986–1994. HAV, hepatitis A virus; *H. pylori, Helicobacter pylori.*

obtained by bacteriological and/or histological examinations; the specificity and sensitivity were 93% and 96.7%, respectively [23, 24]. Anti-HAV antibodies were also assayed by ELISA.

Statistical analysis. The detection of anti–*H. pylori* and/or anti-HAV antibodies was defined as a positive ELISA result. We reassayed all serum samples obtained from participants whose baseline (i.e., 1986) antibody seroprevalence was different. The age-specific seroprevalence of *H. pylori* and HAV antibodies among participants in 1986 and 1994 was analyzed separately. The acquisition of *H. pylori* or HAV was defined as *H. pylori* or HAV antibody seroconversion, respectively. Each participant was observed longitudinally beginning on the date on which serum samples were obtained. The date of acquisition of *H. pylori* or HAV infection, respectively, was defined as the date on which the first positive ELISA results for *H. pylori* or HAV antibodies was recorded. The incidence of either infection (i.e., seroconversion) was computed as the number of new cases in an evaluation period divided by the number of cohort members still at risk. The probability of coincidence of the 2 infections in any single individual was also examined. The κ statistic was used to measure the agreement between seroprevalence of *H. pylori* and HAV infections. The data were analyzed using SAS software (SAS Institute) [25].

RESULTS

Prevalence of antibodies to* H. pylori *and HAV in relation to age and sex: 1986 and 1994 cross-sectional studies. The distribution of the total cohort studied throughout the study period is shown in figure 1. Two independent populations participated in the 2 cross-sectional studies conducted in 1986 and 1994. The age-specific prevalence of both infections increased with age among both study groups, and there was a shift toward lower prevalence in 1994 than in 1986 of *H. pylori* infection and HAV infections. In the 1986 cross-sectional study, there was a significant difference in the overall prevalence of *H. pylori* infection and HAV infection among adults (80% vs. 70%; $P < .001$). Children aged 5–19 years also had a significantly higher prevalence of *H. pylori* infection than HAV infection (31% vs. 5%; $P = .001$). This pattern was consistent in the 1994 cross-sectional study (figure 2). There was no significant difference in the overall prevalence of both infections among males and females in the 2 study periods.

Concordance of* H. pylori *and HAV infections in the same individuals. Of the total 649 participants in the 1986 study, 614 subjects were tested for both *H. pylori* and HAV. Cross-tabulation showed that the probability of concordance of seropositivity for both infections in any single individual was 64% (74.5% among adults and 2% among children). There were 82 individuals (13%) who were seronegative for *H. pylori* and HAV, 99 (16%) who were seropositive for *H. pylori* only, and 39 (6%) who were seropositive for HAV only. Analysis with the κ statistic revealed that the level of agreement between *H. pylori* and HAV seropositivity was better than chance ($\kappa = 0.4$), and, among those aged <20 years, it was worse than chance ($\kappa = 0.05$).

Acquisition and persistence of* H. pylori *and HAV antibodies. There were 480 subjects who were followed-up annually through 1994, 86 of whom were children and 394 of whom were adults (figure 1). The mean ($\pm$SD) follow-up duration for the total cohort included in the analyses was 5.5 $\pm$ 2.7 years and was identical for adults and children, as well as for male and female subjects. Of the 87 individuals who were seronegative for *H. pylori* at study entry in 1986, 8 (9%; 3 adults and 5 children) became infected by the end of the follow-up period in 1994. Table 1 shows the total number of study subjects who had seroconversion, the age at study entry, the number of years to seroconversion. The annual *H. pylori* seroconversion rate (i.e., incidence rate) was calculated on the basis of the assumption that these rates were equally distributed throughout the mean ($\pm$SD) observation period of 5.6 $\pm$ 2.7 years (5 years among children and 6.7 years among adults). The crude rate of *H. pylori* seroconversion was 1.5% of the participants per year (1.2% of adults and 1.8% of children per year). All *H. pylori*–seroconverted children were HAV seronegative throughout the 9-year study period, whereas 2 of the 3 *H. pylori*–seroconverted adults were HAV seropositive during the study period. Of interest, none of the individuals who were seronegative for HAV at study entry became HAV seropositive at any time during the study period.

DISCUSSION

Although childhood is recognized to be the time of high risk for *H. pylori* acquisition [26–29], the definite mode of transmission of *H. pylori* infection has yet to be established. *H. pylori* infection is known to cluster in families [30–32], and the *H. pylori* strains within 1 family are closely related [33]. However, it remains unclear whether transmission is more often due to a common exposure source or by fecal-oral or gastric-oral routes. Previous studies examined the associations of the presence of both HAV and *H. pylori* infections in a population in the attempt to define the mode of transmission of *H. pylori* infection [13–19]. The current study is the first longitudinal study to have compared the seroepidemiologic pattern of HAV and *H. pylori* infections at 2 different time periods in the same community and that examined the seroepidemiologic changes of both infections in the same individuals during a 9-year period.

HAV transmission is known to occur via fecal-oral routes, and it is usually associated with overcrowding, poor hygiene, and unsanitary conditions [34, 35]. Socioeconomic status is also known to be a major risk factor that correlates with the variation in the prevalence of *H. pylori* infection [1–3, 36, 37].

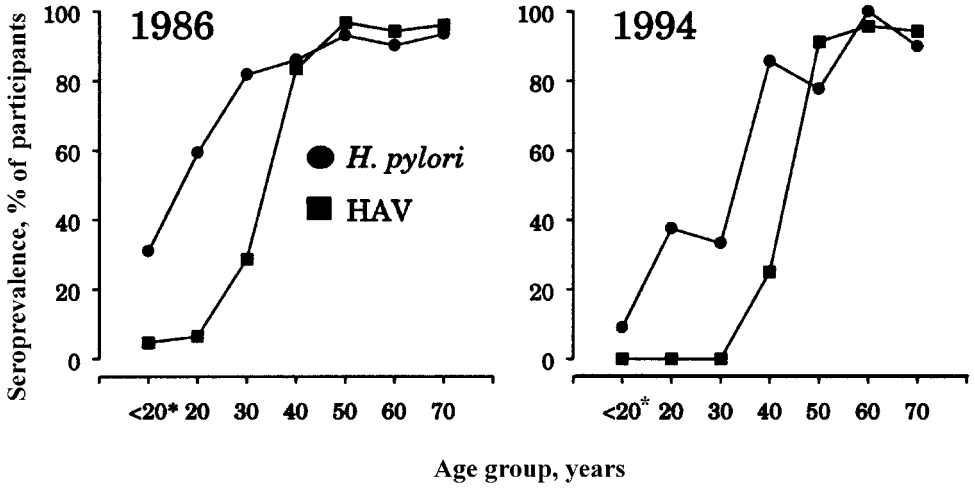

Figure 2. Comparison of the seroepidemiologic patterns of *Helicobacter pylori (H. pylori)* and hepatitis A virus (HAV) infections in 1986 and 1994. *5–10 years of age: seroprevalence of *H. pylori* HAV was 13% and 0%, respectively; 11–19 years of age: seroprevalence of *H. pylori* and HAV was 46% and 8%, respectively.

Our results revealed that, in 1986, there was a high seroprevalence of *H. pylori* and HAV infections in rural Japan, with a strong association with age. That parallel increase in the seroprevalence of HAV and *H. pylori* infections with age reflects a cohort effect that was demonstrated by the lower rates of seroprevalence of both infections among the younger population in the 1994 study. These results represent a cohort phenomenon, because hygienic conditions improved over time in the studied population. There was a rapid change in sanitary conditions and standard of living in Japan after World War II, and clean public water systems were introduced in Japan in the 1950s. The decrease in the seroprevalence of both HAV and *H. pylori* infections from 1986 through 1994 in Japan is consistent with the continuing decrease in the prevalence of both infections in industrialized countries and is most compatible with the hypothesis that both infections are transmitted via fecal-oral routes. It would seem unlikely that there was a major change in the frequency of gastric-oral exposures during this period, although previous studies have reported the possible transmission of *H. pylori* infection through contact with vomit [38–40].

Table 1. Data for data participants who underwent *Helicobacter pylori* seroconversion during the 9-year study period.

Age, years	Follow-up duration, mean years	Interval between study entry and seroconversion
Children	5	
6		3
7		6
8		3
10		5
12		8
Adults	6.7	
24		3
35		8
58		9

Overall, the results of our study are most compatible with the hypothesis that both HAV and *H. pylori* are transmitted via fecal-oral routes but that they are not linked directly, because HAV infection is an acute infection that typically occurs in epidemics, whereas *H. pylori* is an endemic infection with numerous occasions for exposure. HAV can maintain its virulence outside the host for a prolonged period, making contaminated water and food frequent vehicles for its transmission [41]. The results of our study revealed an *H. pylori* seroprevalence of 9% versus an HAV seroprevalence of 0% among the younger population during the 1994 study. Such findings indicate that HAV had spread by means of an outbreak or epidemic that occurred in the area in earlier year(s), whereas *H. pylori* had spread sporadically. The fact that the *H. pylori* strains within families are most often related is consistent with the notion that *H. pylori* transmission is facilitated by interpersonal contact.

The results of the current study are inconsistent with the hypothesis that HAV and *H. pylori* shared a common vehicle for transmission in this population, because none of the children who underwent seroconversion and became *H. pylori* positive also acquired HAV during the 9-year study period. In addition, closer analysis of the data showed that the concordance of the 2 infections in any single individual was lacking, especially among children ($\kappa = 0.05$).

Nevertheless, 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 both *H. pylori* and HAV infections, such as lower socioeconomic status of or crowded living conditions for the studied individuals. However, the study location of South Kiso town provided an opportunity to evaluate the transmission of *H. pylori* infection in a nonaffluent Japanese population without markedly different socioeconomic classes. Second, the small number of individuals tested for HAV and *H. pylori* infection in the 1994 study limited the power to examine the concordance pattern of both infections among the same individuals and to compare it with that revealed in the 1986 study.

In conclusion, this study demonstrated that both *H. pylori* and HAV infections are associated with poor sanitary and hygienic practices, which improved over time in Japan, and provided evidence that both infections are transmitted via fecal-oral routes but are not from a common environmental source. It is likely that different mechanisms are used by *H. pylori* and HAV to escape from a source or reservoir and be conveyed to and enter a susceptible host.

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