ALIMENTARY TRACT

Helicobacter pylori infection in healthy people: a dynamic process?

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Abstract

Epidemiological studies using serological tests have shown that a large proportion of healthy people have antibodies against *Helicobacter pylori* (anti-Hp). It is uncertain whether the presence of anti-Hp indicates active infection or only past exposure to the micro-organism. In this study we determined anti-Hp with a specific enzyme linked immunosorbent assay in 100 healthy volunteers who were at the same time investigated for active *H pylori* infection by means of the ¹³C-urea breath test. Forty nine per cent had a high anti-Hp titre, but only 24% had active *H pylori* infection. Our study suggests that a considerable number of healthy people previously infected with *H pylori* have spontaneously eliminated this micro-organism. We suggest that the inability of ulcer patients to eliminate *H pylori* may be important in the pathogenesis of peptic ulcer disease.

Colonisation of the gastric mucosa by *Helicobacter pylori* is associated with antral gastritis, peptic ulcer disease, and non-ulcer dyspepsia,¹ but the role of *H pylori* in the pathogenesis of peptic ulcer disease remains unclear.²⁻⁸ The methods available for the detection of *H pylori* infection are microbiological culture, histology, and quick urease tests, which all depend on endoscopy and antral mucosal biopsies. Alternatively, *H pylori* infection can be detected non-invasively with the urea breath test and with serology. The urea breath test indirectly shows the urease activity of *H pylori* in the stomach of infected people.⁹⁻¹⁰ ¹³C-labelled urea ingested orally is hydrolysed in the stomach when *H pylori* is present, yielding ¹³CO₂ which can subsequently be quantitated in the breath. Thus the urea breath test is an elegant, indirect method for detecting gastric *H pylori* colonisation. Serological testing is an indirect method that has been extensively used to show *H pylori* infection.¹¹⁻¹⁶ At present it is not clear whether in the large proportion (up to 50%) of healthy people with antibodies to *H pylori* (anti-Hp) there is active colonisation of the gastric mucosa by *H pylori* or whether the antibodies merely represent past exposure to the micro-organism.

The excellent sensitivity and specificity of the urea breath test^{9-10, 17, 18} and its simplicity make it an ideal method for screening people for *H pylori* colonisation. Thus we evaluated 100 healthy

adults with a broad age range for past or active *H pylori* infection using serology and the urea breath test.

Patients and methods**PATIENTS**

One hundred people aged 20 to 92 participated in this study. All were healthy and were invited by the investigators to participate. They were recruited from family members, friends, and relatives of the investigators and fellow workers. None had a history of chronic dyspepsia, peptic ulcers, or gastric or other abdominal surgery except for appendicitis; none had taken antacids, a bismuth preparation, or other drugs for dyspepsia within the previous year, and none had taken antibiotics within the three months before the study. The protocol was approved by the University of Basel Ethical Committee and each subject gave written informed consent.

¹³C-UREA BREATH TEST

All breath tests were performed in the morning after an overnight fast according to the method used and validated previously¹⁰; Dill *et al* have shown that the urea breath test has a sensitivity and specificity of 97% and 100%, respectively.¹⁰ Briefly, the subjects drank 150 ml of a liquid test meal to delay gastric emptying and subsequently 150 mg ¹³C-urea dissolved in 20 ml of tap water. ¹³C-urea (99% ¹³C) was purchased as a crystalline substance (Tracer Technologies Inc, Somerville MA, USA). The test meal we used was a 25% glucose polymeric solution that was prepared as a powder (glucose 4 g, maltose 2 g, polysaccharide 30 g, citric acid 0.2 g) in 250 ml bottles and dissolved with 150 ml of tap water immediately before use. Two breath samples were obtained at baseline and then every 15 min for up to 60 min and stored in silicon-coated, evacuated 20 ml glass tubes (vacutainers) for later analysis. Excess ¹³CO₂ in breath samples was measured by isotope ratio mass spectrometry (Sira 10, VG Instruments GmbH, Wiesbaden, Germany). The results of the breath test are expressed as the percentage of the ¹³C dose recovered in breath over one hour (area under the curve (AUC)=% dose/60 min). The breath test was defined as positive if the AUC exceeded 0.5% dose/60 min.

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Figure 1: Results of the ^{13}C -urea breath test in 51 men (A) and 49 women (B) by age. AUC=area under the curve.

ENZYME LINKED IMMUNOSORBENT ASSAY (ELISA) TEST FOR *H. pylori* ANTIBODIES

Sera were tested for IgG antibodies against *H. pylori* (anti-Hp) with a specific ELISA as previously described.¹⁶ Briefly, the antigen was prepared from stationary phase cultures of *H. pylori* strain Li 1103 that were suspended in phosphate buffered saline (PBS, pH 7.0), freeze thawed (2x), ultrasonicated for 60 s (B12 sonifier, set at 70) (BronSonic, Bender Hobein AG, Zurich, Switzerland), pelleted at $1.2 \times 10^4 \text{ g}$ for 10 min, resuspended in PBS, and stored in aliquots at -80°C . For the ELISA the antigen preparation was diluted with coating buffer to yield a final protein concentration of $1.3 \mu\text{g/ml}$; 0.1 ml was dispensed into each well of flat bottomed microtitre plates (Immulon MI 29A, Dynatech Laboratories Inc, Alexandria VA, USA) and kept at 4°C for 16 hours. The sera, including a negative and a pooled high titre positive control serum, were added to the wells by serial threefold dilutions with PBS (from 1:40 to 1:87 480). The plates were incubated for two hours at 37°C , washed with an automatic washer (Dynawasher 2, Dynatech Laboratories Inc, Alexandria VA, USA), and incubated at 37°C for one hour with gamma-chain specific, 1:1000 diluted, goat antihuman IgG antiserum conjugated with alkaline phosphatase (Tago Inc, Burlingame CA, USA). After washing, p-nitrophenylphosphate (Sigma Chemie GmbH, Deisenhofen, Germany) was added and extinction read after one hour at 405 nm (Titertek Multiscan MC, Flow Laboratories Co Ltd, Baar, Switzerland). The specificity of anti-Hp antibodies was assessed with multiple absorptions of a rabbit antiserum against *H. pylori* Li 1103 and a high anti-Hp titre human serum pool with a variety of living bacteria, including *Escherichia coli* (ATCC 25922), *Streptococcus sanguis* (Li 1345), *Salmonella typhimurium* (ATCC 13311),

Figure 2: Relative percentages of healthy subjects with active *H. pylori* infection as indicated by a positive ^{13}C -urea breath test (UBT) and with serological evidence of past or active infection as indicated by anti-Hp antibodies. The number of persons tested in each age group is given in parentheses.

Campylobacter jejuni (Varia 85A), *Campylobacter fetus* (K 1578), *H. pylori* (K 1585), and *H. pylori* (Li 1103). Antibodies were almost completely removed by the two *H. pylori* strains, but not absorbed by any of the other micro-organisms tested.

The results are expressed as antibody titres, defined as the reciprocal values of the serum dilution at the intercept of the respective dilution curves with the baseline.¹⁹ The baseline was defined as extinction measured for the positive control serum at the dilution 1:9720. We have previously shown that a titre of 800 best separated people with and without *H. pylori* infection.¹⁶ In the present study we have therefore classified people as having anti-Hp if their titre exceeds 800.

STATISTICAL ANALYSIS

H. pylori infection was assessed with serology and the urea breath test. Because the two parameters are interrelated the proportions of people with positive test results were compared with

TABLE I Anti-Hp antibody titres in 100 healthy subjects with positive or negative urea breath tests (UBT)

	Anti-Hp titre >800		Anti-Hp titre <800 and UBT negative
	UBT positive	UBT negative	
No	24 (11 M, 13 F)	25 (10 M, 15 F)	51 (30 M, 21 F)
Median	8586	1741*	218
Range	841-78 918	804-29 160	40-756

* $p < 0.001$, significantly lower in comparison to UBT positive group.

TABLE II Results of serology and urea breath test (UBT) in different age groups shown as number with positive results/number tested

Age group (years)	Anti-Hp titre >800	UBT positive
20-29	5/20	2/20
30-39	8/16	3/16
40-49	11/20	4/20
50-59	8/14	6/14
60-69	7/15	2/15
≥70	10/15	7/15
Total	49/100	24/100*

* $p < 0.0001$.

McNemar's test for dependent samples.²⁰ Antibody titres between different groups were compared with the Mann-Whitney U test.

Results

Forty nine of the 100 healthy subjects had anti-Hp titres exceeding 800 (21 men, 28 women). Only 24 of these 49, a significantly ($\chi^2=18.62$, $p<0.0001$) lower proportion, had a positive urea breath test (11 men, 13 women; Fig 1A and B). The other 25 subjects (10 men, 15 women) had anti-Hp only and negative urea breath test results. Their titres were significantly ($p<0.001$) lower than those of the urea breath test positive subjects (Table I). In 51 of the 100 subjects (30 men, 21 women) there was no sign of *H. pylori* infection since they had no anti-Hp and negative urea breath test results (Table I). There was a tendency for both tests to be positive more frequently in elderly subjects (aged over 50 years). The prevalence, however, of anti-Hp positive subjects exceeded that of urea breath test positive subjects in all age groups (Fig 2). There was no difference between men and women. Furthermore, among the six gastroenterologists who participated in this study only two had *H. pylori* infection as shown by positive urea breath test and serology. In the remaining four both tests were negative.

Discussion

Little is known about the prevalence of *H. pylori* infection in the general population with no gastrointestinal symptoms. The results of our study show that 49% of a representative sample of the adult Swiss population²¹ have anti-Hp, but only 24% have active *H. pylori* infection as indicated by a positive urea breath test ($p<0.0001$). Thus 50% of previously infected healthy people with anti-Hp may have spontaneously eliminated *H. pylori*.

Our findings are based on two indirect tests. The conclusion, therefore, depends on the specificity and sensitivity of the antibody determination and the urea breath test, respectively. In the present study we classified people as anti-Hp if their titre was above 800. Similar ELISA titres have been found previously to separate people reliably with and without *H. pylori* infection.¹² The prevalence of anti-Hp positive healthy subjects found in this study agrees closely with what has been reported: anti-Hp increases with age and occurs in up to 50% of people aged 60 and older.^{1 10 11}

Only 24 out of 49 subjects with high anti-Hp titres had a positive urea breath test and thus active *H. pylori* infection. Twenty five had anti-Hp only and negative urea breath test results. In the studies that have shown that the urea breath test reliably separates patients with and without gastric *H. pylori* colonisation^{9 16-18} the sensitivity of the urea breath test exceeded 97% in all of them; among 34 untreated subjects with proved *H. pylori* infection, a false negative urea breath test result occurred in just one.¹⁰ The prevalence of active *H. pylori* infection was previously investigated in asymptomatic volunteers willing to undergo endoscopy.^{2-6 22} In the largest

of these studies²² *H. pylori* infection was proved by a positive culture in 19.4%, whereas serology was positive in 33.7%. Graham *et al* tested healthy volunteers with the urea breath test and reported positive results in 45%²³; the antibody state of the participants was, however, not determined.

The results of our study imply that a substantial proportion of healthy people who were infected with *H. pylori* must have eliminated this micro-organism. This phenomenon may have been observed before, but has received little attention. Musgrove *et al* reported that eight of 39 patients without gastritis and without *H. pylori* had high anti-Hp titres¹³ and Loffeld *et al*, in a similar study, found that six of 22 patients without *H. pylori* had anti-Hp titres in the range found in actively infected patients.¹⁴ Dent *et al*, using a different serological test, found high titres in 29 patients in whom *H. pylori* was not detected, and although most of them had gastritis, there were still 10 of 75 patients without gastritis who had positive titres.¹⁵ All of these results support our hypothesis that *H. pylori* colonisation is a dynamic process with an active phase of infection and subsequent elimination of the micro-organism in at least a proportion of infected people.

Our study may have some relevant clinical and pathophysiological implications. It has been suggested recently that serology might replace endoscopy in the diagnosis of *H. pylori* associated gastritis.¹⁴ Our study clearly shows that the presence of anti-Hp does not indicate whether a person is actively infected or not.

Finally, we would like to develop a hypothesis which might have relevance for the pathogenesis of peptic ulcer disease. We suggest that healthy people may remain so because they are able to eliminate *H. pylori*, whereas those who will develop ulcers have a defect in the gastric mucosa that makes it susceptible to *H. pylori* colonisation and in turn vulnerable to the acid attack. These patients are unable to eliminate *H. pylori* spontaneously and they remain susceptible to infection with *H. pylori* even after treatment with antibiotics. Thus recurrence or reinfection may take place and be responsible for the high ulcer relapse rates.⁸ This hypothesis needs to be tested by sequential investigation of healthy people. The ¹³C-urea breath test safely permits repeated examinations and is therefore suitable for long-term follow up of healthy people to show the incidence of *H. pylori* infection and the proportion of infected people that will spontaneously eliminate this micro-organism.

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