Helicobacter pylori reinfection with identical organisms: transmission by the patients' spouses

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Abstract

Reinfection with *Helicobacter pylori* after eradication is responsible for the recurrence of duodenal ulcer disease. The mode of transmission has not yet been established. In this study, 18 patients with chronic duodenal ulcers in whom *H pylori* had been eradicated with amoxicillin and metronidazole were entered into a prospective follow up study. Control endoscopies were performed 4, 8, 14, 27, and 43 months after starting treatment and the results of direct tests were compared with the kinetics of *H pylori* specific IgG titres. After eradication there was a noticeable and consistent fall in anti-*H pylori* IgG, while reinfections were characterised by a significant increase in specific titres. Reinfection was detected in two patients after 14 and 43 months, respectively. The *H pylori* strains responsible for these reinfections, the corresponding pretreatment isolates, and the strains isolated from the spouses of these patients were examined by polymerase chain reaction based DNA fingerprinting. Analysis showed that reinfection had been caused by the same *H pylori* strain and identified the spouses of these patients as carriers of the identical strain. Considering the genomic diversity and the interpatient heterogeneity of *H pylori* these results suggest a person to person transmission of *H pylori* reinfection. By the end of the observation period reflux oesophagitis had developed in 10 of the 16 patients who had not been reinfected. This surprising finding may be explained by the changed eating habits of patients after healing of duodenal ulcer disease.

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Duodenal ulcer disease can be cured by eradication of *Helicobacter pylori*,[1] but reinfection may occur and ulcer relapse rates of up to 11·7% have been reported.[2 3] To determine the possible mode of reinfection, we followed patients with eradicated *H pylori* for 43 months after eradication. The objective of this prospective follow up was to investigate the frequency and the source of reinfection. A second objective was to evaluate the correlation between serology and invasive tests in detecting *H pylori* reinfections as varying reinfection rates have been described with different types of detection. While low rates have been reported with uninvasive methods, higher rates have been observed with direct tests.[3 4]

Patients and methods

H pylori eradication in subjects with a history of at least two symptomatic recurrences and an endoscopically confirmed, active duodenal ulcer was achieved with a 12 day regimen of amoxicillin (750 mg three times daily) plus metronidazole (500 mg three times daily), already described elsewhere.[5] Additional ranitidine was given for six (or 10) weeks. After ulcer healing and *H pylori* eradication, control endoscopies were performed on months 4, 8, 14±1, 27±2, and 43±4 after starting treatment or whenever symptoms occurred. If reinfection was detected, the spouse of the reinfected patient was also asked to undergo endoscopy.

H PYLORI STATUS

At each endoscopy five biopsy specimens were collected from the antral mucosa. *H pylori* was identified by the rapid urease test, histological examination, and culture, as already described previously.[5] The organism was considered present, if all three tests were positive. It was considered to have been eradicated, if negative results were obtained in all of the three tests. (In our investigations, diverging results for the three tests were never seen.)

SEROLOGY

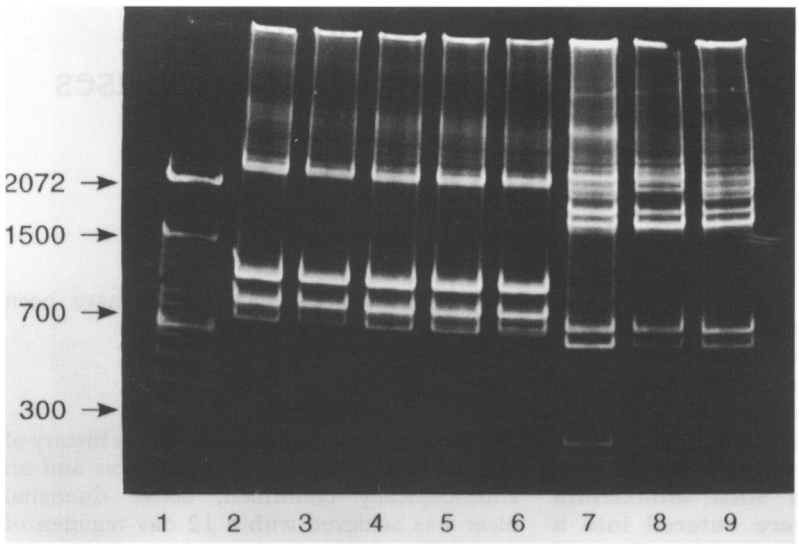
Sera were stored at −20°C until use. The specific IgG titres were determined by a fluorescence enzyme immune assay (Heloritest IgG, Eurospital, Italy). The amount of specific antibodies in each sample was expressed as an index % obtained by comparing the patient's value with that of the positive control according to the following formula:

$$\text{INDEX \%} = \frac{\text{fluorescence units sample}}{\text{fluorescence units positive control}} \times 100$$

All sera from each individual patient were tested in duplicate at the same time.

H PYLORI TYPING

H pylori strains were stored at −70°C in 0·5 ml horse serum +17% glycerol. If a reinfection occurred, the newly detected strains were compared to the corresponding pretreatment isolates. In addition, the isolates of the reinfected patient's spouse were obtained at the end of the follow up period. Typing was



Ethidium bromide staining of AP-polymerase chain reaction products of several H pylori *strains separated by 4–20% polyacrylamide electrophoresis. Lane 1=molecular weight marker (bp); 2=patient L before onset of treatment; 3=patient L at month 14 (reinfection); 4=patient L at month 27; 5=patient L at month 43; 6=spouse of patient L; 7=patient K before onset of treatment; 8=patient K at month 43 (reinfection); 9=spouse of patient K.*

performed using polymerase chain reaction (PCR) based DNA fingerprinting. Arbitrary primer (AP) PCR was used to detect any DNA sequence diversity among the *H pylori* isolates. The *H pylori* DNA was prepared by repeated freezing and thawing of a suspension of *H pylori* in phosphate buffered saline. Phenol/chloroform extraction, precipitation of DNA and PCR were performed as described by Akopyanz *et al.*[6] For PCR a 10nt-primer 5′-CCGCAGCCAA-3′ was used.

Results

Eighteen patients (14 men and four women with a mean age of 50 years) with healed duodenal ulcers and eradicated *H pylori* were entered into the follow up study.

SEROLOGY AND DIRECT TESTS

The median pretreatment IgG index was 89·9%. The specific IgG titres showed a continual decrease with a median IgG index reduction of 66% up to month 14, thereafter the titres of the patients who had not been reinfected remained at a low level until the end of the follow up period. In these patients, urease tests, histological examinations, and bacteriological cultures yielded negative results at each of the controls. In patient L reinfection was detected at the 14 month check. All direct tests had become positive again and the IgG index approached the pretreatment level, falling short of this value by only 0·2%. The reinfection of patient K was diagnosed at month 43. All invasive tests yielded positive results, while the IgG index actually exceeded the pretreatment value by 17·3%.

H PYLORI TYPING

As illustrated in the Figure, the *H pylori* strains found in patient L before the onset of treatment and at the post reinfection controls at months 14, 27, and 43, as well as those isolated from the patient's spouse, were completely identical. In patient K, the strains isolated before treatment and those found after reinfection at month 43 were virtually identical, while the strain isolated in the patient's spouse was identical to the patient's pretreatment strain.

ENDOSCOPIC AND CLINICAL FINDINGS

Patient L presented with mild symptoms at month 14, but endoscopy did not show any ulcer recurrence. Since the patient's symptoms persisted and even worsened during the second follow up year, four additional endoscopies were performed, but failed to show an ulcer. Patient K complained about mild symptoms at the 43 month control, when reinfection was detected. However, no ulcer recurrence could be found. The spouses of both patients were free of any symptoms. For the remaining 16 patients who had not been reinfected, no ulcer relapses could be seen and they continued to be free of related symptoms. In one of these patients reflux oesophagitis was diagnosed at the control after 14 months. After 27 months of follow up, three of the patients had developed reflux oesophagitis, a finding which was deemed without particular significance by the investigators. At the end of the follow up period, however, reflux oesophagitis (Savary-Miller grade 1) was seen in 10 of those who had not been reinfected. Most of them had not spontaneously complained about their reflux symptoms because they had not associated the mild heartburn experienced with their ulcer disease.

Discussion

Since *H pylori* shows a high degree of genomic diversity most patients carry an individual strain with a unique pattern.[7] As shown by Bamford *et al*, different family members may be infected with the same strain, but usually typable strains differ significantly from patient to patient.[6 8 9] For cases where strains with an identical DNA pattern can be detected before eradication and after *H pylori* reinfection, two principal explanations may be suggested as follows:

(i) A relapse – that is the organism was not completely eradicated, but only transiently suppressed. This is highly unlikely in our two cases, because *H pylori* was only redetected at 14 and 43 months respectively after the onset of treatment.

(ii) Reinfection with the same strain; this would be possible by transmission from family members or spouses infected with the identical strain.

The data available on the person to person spread of these bacterial organisms are rather inconsistent. Malaty *et al* report a high incidence of *H pylori* infections in spouses of *H pylori* positive patients and intrafamilial clustering of *H pylori* infections has been described by Drumm *et al.*[10 11] In obvious

contrast, Perez-Perez *et al* suggest that person to person transmission does not occur at all, or at least only infrequently.[12] The results of all these studies are exclusively based on serological data and did not involve any DNA analyses. In our investigations, no genomic diversity could be seen for the isolates of the spouses and those of the reinfected patients. With the identification of identical strains in both the healthy *H pylori* carrying spouse and the reinfected patient, conjugal transmission in reinfection has been shown for the first time with near certainty. A common exogenous source of *H pylori* cannot be ruled out altogether, but seems rather unlikely.

Reinfection rates have generally been reported to range between 0·48 and 35·3% in one year.[3 13] While the reinfection rates described for investigations based on non-invasive methods are similarly low as those for seroconversion studies, endoscopic follow up usually shows higher rates.[3 14–19] In our study, a reinfection rate of 3·1% per patient year was seen, with excellent correlation between serological evidence of reinfection and its demonstration by microbiological and histopathological methods.

To our knowledge, the development of reflux oesophagitis after *H pylori* eradication has never been reported. We do not believe that this was an accidental finding or that reflux oesophagitis escaped the attention of the experienced endoscopists at the preceding gastroscopies. We think that its development was caused by the changed eating and drinking habits of patients after the healing of their duodenal ulcer disease. The patients were no longer bound to any dietary regimen and all of them showed an obvious weight gain. Upon inquiry, they reported having been able to enjoy acid producing foods and those that reduced lower oesophageal sphincter pressure and alcoholic beverages after *H pylori* eradication.

In conclusion, these results provide evidence that reinfection with *H pylori* may be caused by the same *H pylori* strain. Serological follow up seems to be sufficient for the detection of reinfection. Person to person transmission is the most likely mechanism and this warrants looking for *H pylori* IgG antibodies in the spouse of a duodenal ulcer patient reinfected with this organism. In the case of positive results anti-*H pylori* treatment of both patient and spouse is well worth considering.

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