Helicobacter pylori infection in spouses of patients with duodenal ulcers and comparison of ribosomal RNA gene patterns

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Abstract

Background—In recent studies, familial coinfection with the same *Helicobacter pylori* strains has been indicated, but more data are necessary to confirm intra-familial spread of the micro-organism.

Aims—The aim of this study was (a) to assess the frequency of *H pylori* infection in spouses of patients with duodenal ulcers and (b) to investigate the possibility of intraspousal transmission of *H pylori* by molecular typing of the respective strains.

Patients—Sixty four patients with duodenal ulcer and their spouses were included in the study.

Methods—The *H pylori* infection was confirmed after endoscopy by culture and histological examination of biopsy specimens, and CLO test. The isolates were compared on the basis of their rRNA gene patterns (ribopatterns) after digestion of chromosomal DNA by the restriction endonucleases *Hae*III or *Hind*III.

Results—Of the patients, 54 were found to be *H pylori* positive. Of the respective spouses, 42 (78%) were also *H pylori* positive. In contrast, only two out of 10 (20%) partners of *H pylori* negative patients were infected. Ribopatterns of *H pylori* strains derived from 18 patients and their spouses showed that in each of eight couples a single strain had colonised both partners, while in the remaining 10 couples each partner was colonised by a distinct *H pylori* strain.

Conclusions—These data suggest person to person transmission within couples or exposure to a common source of infection.

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Helicobacter pylori has been associated with the pathogenesis of chronic active gastritis and peptic ulcer disease.^{1 2} There is also evidence that the micro-organism is implicated in the development of gastric carcinoma.³ In spite of the numerous relevant studies, several epidemiological characteristics of the *H pylori* infection such as the reservoirs of the micro-organism and the exact mode of its transmission have not yet been clearly defined. Previous seroprevalence studies have shown that *H pylori* infection is mainly acquired during childhood and is strongly related to socio-

economic factors.^{4–6} However, some seropositivity occurs slowly in adults,⁵ suggesting that acquisition of *H pylori* might also take place during adulthood. More details on the epidemiological features of *H pylori* infection have been obtained by the application of molecular typing techniques, which allow efficient discrimination between *H pylori* strains because the micro-organism exhibits an extensive genetic heterogeneity. With the aid of the above techniques, infection with the same *H pylori* strain in people that are in close contact, such as institutionalised children and members of the same family, has been clearly demonstrated in several recent reports.^{7–10} The above findings, along with relevant seroepidemiological data, may indicate that a direct person to person transmission does occur and being in contact with an *H pylori* infected person increases the risk of being infected. In this study we attempted to analyse some epidemiological aspects of *H pylori* infection in spouses of patients with duodenal ulcer (DU) disease and compare, when available, the DNA fingerprints of the isolated *H pylori* strains by ribotyping.

Patients and methods

Study population

From late 1992 until the end of 1994, 95 married patients with DU confirmed by endoscopy and their spouses were asked to participate. The patients were consecutive and originated from the Athens area and various parts of central and south Greece. Seventy two couples gave informed written consent in accordance with the guidelines of the Committee on Human Investigation. The following patients were excluded: (1) those with other present or recent serious diseases, or a history of intervention of the upper gastrointestinal tract, (2) those who had received antibiotics or bismuth treatment in the two months before enrolment or (K⁺-H⁺)-ATPase inhibitors two weeks before enrolment and (3) pregnant women. Sixty four patients with DU and their spouses fulfilled the inclusion criteria, completed a questionnaire on age, socioeconomic status, symptoms from the upper gastrointestinal tract, and the use of non-steroid anti-inflammatory drugs (NSAIDs).

Endoscopy and diagnosis of *H pylori* infection

Endoscopy was performed after an overnight fast. The gastroscopes and biopsy forceps were

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*Deceased. This paper is
dedicated to his memory.

thoroughly cleaned with neutral detergent and glutaraldehyde (2.3%, v/v) between endoscopies. A single biopsy forceps was used for each individual. Eight biopsy specimens were taken from each participant. Six were from the antrum and two from the corpus. Two of the antrum specimens and the two corpus specimens were exclusively used for histological examination, two were used for culture and two used for the CLO test. Patients were considered to be *H pylori* positive if they produced positive results with at least two methods. Patients were considered as *H pylori* negative if all tests gave negative results.

For histological examination, biopsy specimens were fixed in formalin and examined for the presence of *H pylori* by a modified Giemsa staining. The Warthin-Starry technique was employed for undetermined cases.

Biopsy specimens were transported in thioglycolate broth and cultured on modified GAB-CAMP medium,¹¹ in a microaerobic atmosphere for up to seven days. Colonies of typical appearance, Gram stain and positive for both catalase and urease production were identified as *H pylori*.

Treatment and follow up

H pylori positive patients were treated with the following regimen: omeprazole (20 mg twice daily) for two weeks, followed by the classic triple therapy that included histacycline (500 mg four times a day), metronidazole (500 mg three times a day) and CBS (300 mg four times a day) for two more weeks. The *H pylori* negative patients and their partners were used as a control population. *H pylori* infected patients with DU who entered the study were reassessed by gastroscopy (at four weeks and 6–12 months after the completion of therapy) for ulcer healing and *H pylori* status. The spouses of patients were subjected to one endoscopic examination, after the second endoscopy of the patient. None of the latter group received any eradication treatment no matter what the *H pylori* status.

DNA typing

Chromosomal DNA samples of *H pylori* isolates derived from 18 patients with DU and their spouses were prepared and partially purified by the guanidium thiocyanate method essentially as described previously.¹² DNA samples were digested with restriction endonucleases *Hae*III or *Hind*III for four hours. The digested DNA was electrophoresed at 25 V for 18 hours in 0.75% (w/v) agarose. After depurination, denaturation and neutralisation, the DNA was transferred to Hybond-C nitrocellulose membranes. After prehybridisation in a mix consisting of 5×SSC (1×SSC is 0.15 M NaCl/0.015 M sodium citrate), 50% formamide, 0.1% Ficoll, 0.1% polyvinylpyrrolidone, 0.1% bovine serum albumin, 0.5% sodium dodecyl sulphate and 500 mg/ml denatured herring sperm DNA, membranes were hybridised with a biotinylated cDNA probe that was prepared from 16+23S rRNA of

Escherichia coli MRE 600 (Boehringer, Mannheim, Germany). Detection was performed using the BluGENE detection kit (Gibco-BRL, Bethesda, MD, USA).

Statistical analysis

Frequency analysis was performed by Fisher's exact test. Odds ratio was estimated using the 95% Taylor series confidence limits.

Results

Sixty four patients (46 males) aged from 28 to 71 (mean age 46.8) years and their respective partners (46 females) aged from 25 to 68 (mean age 43.2) years were assigned to statistical analysis. Fifty four patients were *H pylori* positive and 10 were negative. Five of the latter were chronic NSAID users. In the remaining five patients the cause of DU remained obscured. Forty two (78%) of 54 partners of *H pylori* positive patients were also *H pylori* positive. In contrast, only two (20%) spouses of *H pylori* negative patients were *H pylori* positive. The frequency thus of *H pylori* infection in the spouses of *H pylori* positive patients with DU (group A) was significantly higher than the frequency observed in the spouses of *H pylori* negative patients with DU (group B) (odds ratio=14.04 2.26<OR<144.64, Fisher's exact test $p<0.00084$). It should be noted, however, that *H pylori* negative spouses of *H pylori* positive patients (group A1) were significantly younger (mean age 37.4 years) than *H pylori* positive spouses of *H pylori* positive patients (mean age 45 years) (group A2) (t test, $p<0.05$). Also, the duration of cohabitation was significantly longer in the latter (mean value 20.3 years) than in the former (mean value 7.4 years) group (t test, $p<0.05$). None of the spouses of patients with DU had the disease except one who had developed duodenal erosions.

H pylori strains were isolated by culture in both the patient and the partner in 26 of 42 couples (62%). Chromosomal DNA from 36 isolates (18 from patients and 18 from the respective spouses) was analysed by comparison of the restriction pattern of the rRNA genes. Comparative analysis of the strains isolated from the remaining eight couples was not possible because of the loss of at least one strain from each pair after subculturing or during storage. Thirty isolates were successfully digested by *Hae*III. Analysis of the ribopatterns of the remaining six isolates was achieved after digestion with *Hind*III. In Figure 1, a diagrammatic representation of the ribotype profiles of the strains examined is shown. In eight couples (44%) both spouses harboured a single *H pylori* strain, as it is evident from the similar ribopattern profiles (Fig 2). In the remaining 10 couples, the spouses harboured *H pylori* strains displaying different ribopatterns. *H pylori* ribopatterns were unique among unrelated individuals.

Overall, in six patients, relapse of DU was observed after 6–18 months. All were *H pylori* positive on histological examination and by the

Figure 1: Diagrammatic representation of rRNA gene patterns of *H pylori* strains. Each letter (A to R) represents one couple, while numbers 1 and 2 represent the strains recovered from the patient with DU and the respective spouse. The chromosomal DNA of *H pylori* strains from couples A, B, D-J and M, N, O and R was digested with HaeIII, while the DNA of *H pylori* strains from couples C, K, L, P and Q was digested with HindIII. Bacteriophage λ DNA digested with HindIII was used as a molecular size marker. Comparisons of the ribopatterns showed that in each of the couples A, D, E, G, J, K, M and R, a single strain had colonised both partners. In the remaining 10 couples, the patients harboured *H pylori* strains displaying ribopatterns different from those of their partners' strains.

CLO test. Cultivation of *H pylori* strains was achieved from the new biopsy specimens derived from three of the above patients and analysed by ribotyping. In the two of them, the ribopatterns were identical with those of the initially isolated strains. Interestingly, the pattern of the strain from the third patient was different from that of the previously isolated strain but it was similar with the ribopattern of the strain isolated from his wife (Fig 3).

Discussion

H pylori colonisation usually is initiated in early childhood with the highest incidence by the age of 15 years.⁴⁻⁶ There is also evidence suggesting

that a percentage of *H pylori* infection is acquired during adulthood.⁵ Since intrafamilial dissemination of *H pylori* clones has been reported,^{7-10 13} we examined the possibility of intraspousal transmission of the micro-organism between cohabiting married couples. In a previous seroepidemiological study it was found that as many as 70% of the adult Greek population possesses anti-*H pylori* antibodies,¹⁴ indicating that intraspousal transmission might be difficult to demonstrate by conventional serological assays. The data presented here show a significantly higher prevalence of *H pylori* infection in spouses of *H pylori* positive patients with DU (group A) than in spouses of *H pylori* negative patients (group B).

Comparison of the rRNA gene patterns of the available *H pylori* strains showed that in 10 couples (56%) each partner was colonised by a distinct *H pylori* strain. Similar *H pylori* ribopatterns were observed between spouses in each of the remaining eight couples (44%). The high degree of the observed similarities indicated clearly that in each of these eight couples a single *H pylori* strain had colonised both partners. It should also be mentioned that the gastric mucosa could be colonised by more than one distinct strain.¹⁵ Therefore, the percentage of couples sharing the same strain might be higher than that observed. The above results show that in a considerable percentage of couples in which one is a *H pylori* positive patient with DU disease, there is a transfer of *H pylori* strains or exposure to a common source of infection. However, the former notion seems to be the more likely because to date no environmental source of *H pylori* has been recognised with certainty and the most probable ecological niche of the micro-organism is the human gastric mucosa. The hypothesis of direct transfer of *H pylori* within couples is further supported by the fact that the strain isolated in one case of relapse was similar, in terms of ribopattern, with that derived from the respective healthy spouse. Also, reinfection of patients, after successful

Figure 2: Ribopatterns of the *H pylori* isolates from eight patients with DU (lanes: A1, D1, E1, G1, J1, K1, M1 and R1) are presented in parallel with the ribopatterns of the similar strains (lanes: A2, D2, E2, G2, J2, K2, M2 and R2) isolated from the respective spouses. In lanes M, λ DNA digested with HindIII are presented. Molecular size is indicated on the left.

Figure 3: Comparison of rRNA gene patterns of *H. pylori* strains isolated from three patients who suffered a DU relapse and their spouses. In lanes B1, H1 and O1, strains from the initial biopsy specimens of the patients are presented. Strains derived from either the biopsy taken after relapse or from the spouse of each patient are presented in lanes B3, H3, O3 and B2, H2, O2 respectively. Molecular sizes are from phage λ HindIII digests (first and last lanes). In patients H and O, the initial strains (H1 and O1) displayed the same patterns with those isolated after relapse (H3 and O3) but different from the strains isolated from their spouses (H2 and O2). In patient B, the strain isolated after DU relapse (B3) differed from the initial strain (B1), while it was similar with that of his wife (B2).

eradication therapy, by *H. pylori* strains harboured by their healthy spouses has recently been suggested.¹⁰ With respect to the direction and the mode of spread of *H. pylori* clones within couples no certain conclusions can be drawn at present. It seems reasonable to hypothesise that the actual source of infection is the *H. pylori* positive patient. As shown by histological examination, most of these patients were heavily colonised by the micro-organism. Thus, mouth secretions may be contaminated from the *H. pylori* in gastric juice and the micro-organism could be transferred through the oral-oral contact of the couple. However, the faecal-oral transmission cannot be excluded. Of the eight pairs of similar *H. pylori* strains, only in one case was a subtypic difference restricted to one band of the *Hae*III digestion pattern observed. The causes of the minor genetic differences between these clonal variants are unknown. They probably reflect a tendency for spontaneous genomic rearrangements or an in vivo selection of mutants.

Of interest is the fact that the *H. pylori* negative partners of *H. pylori* positive patients were younger and the duration of their cohabitation was shorter than the *H. pylori* positive partners of *H. pylori* positive patients. As already mentioned, the correlation between age and *H. pylori* infection has been established in several studies. Although it could be argued that duration of cohabitation is included in the factor 'age', it might act, at least partly, independently as a risk factor for *H. pylori* colonisation. This aspect is compatible with

our finding that in eight couples the partners share the same *H. pylori* strain.

On the basis of molecular typing data, it has been suggested that there are disease specific *H. pylori* strains,^{16, 17} whereas other investigators report that ulcer development is irrespective of the colonising strain.^{18, 19} The results of this study favour the latter suggestion since none of the eight spouses infected with *H. pylori* strains identical with those isolated from the respective patients with DU was dyspeptic or had signs of ulceration. Moreover, the strains isolated from three individuals who reported dyspepsia (one also had duodenal erosions) were different from the strains derived from their respective ulcerated spouses. It must be noted, however, that whether or not ulcerogenic *H. pylori* strains exist, the possibility that development of ulceration after colonisation depends, at least partly, on the genetic make up of the host.²⁰

Overall, the data presented here support the suggestion that spouses of *H. pylori* positive patients with DU constitute a high risk group for colonisation from *H. pylori* and subsequent development of either duodenal or gastric ulcer disease. To test further this hypothesis we have initiated a follow up of the *H. pylori* positive healthy individuals detected in the present study.

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