Rare Incidence of Interspousal Transmission of Helicobacter pylori in Asymptomatic Individuals in Japan

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PCR-restriction fragment length polymorphism electrophoretic patterns of amplified ureB and ureC of Helicobacter pylori were compared between spouses after digestion with restriction endonucleases. Twenty of 21 couples, both members of which were positive for H. pylori, showed ureB and ureC patterns that differed between spouses. We concluded that in Japan, interspousal transmission of H. pylori occurs rarely.

Although previous studies have suggested the spread of Helicobacter pylori by close human contact (5), the major mode of transmission of this bacterium is still uncertain. Recently, some papers (4, 10) noted the possibility of interspousal transmission of H. pylori. Therefore, the present study was conducted to investigate the prevalence of interspousal transmission of H. pylori in asymptomatic couples in Japan using PCR-based restriction fragment length polymorphism (RFLP) analysis.

Seventy volunteer couples with no recent history of antibiotic use were enrolled in this study and, after giving informed consent, underwent endoscopic examinations at the University of Tokyo Hospital. At each endoscopic examination, several biopsy specimens were taken from the antrum and body of the stomach for culture. Each biopsy specimen was placed in a Seed Tube HP (Eiken Chemical Co. Ltd., Tokyo, Japan) and was included to ensure full extension of the product. To examine the homogeneity and yield of the target DNA extracted and 50 cycles of PCR amplification was performed with 50-μl reaction mixtures containing 5 μl of 10× PCR buffer, 4 μl of a deoxynucleotide triphosphate mixture (2.5 mM each dATP, dCTP, dGTP, and dTTP; Takara Shuzo Co. Ltd., Shiga, Japan), 0.25 μl of each oligonucleotide primer, 0.25 m of AmpliTaq DNA polymerase (5 U/ml; Hoffmann-La Roche, Inc., Nutley, N.J.); and 5 μl of DNA which had been extracted by the phenol-chloroform method (2) using H. pylori obtained from biopsy specimens. The reaction conditions included initial denaturation of the target DNA extracted and 50 cycles of 94°C for 30 s, 55°C for 1 min, and 72°C for 1 min. The final cycle was 7 min at 72°C and was included to ensure full extension of the product. To examine the homogeneity and yield of the ureC and ureB amplifiers, 2 μl of the PCR product was electrophoresed on a 3% agarose gel with 1× Tris-acetate-EDTA buffer containing 0.5 μg of ethidium bromide per ml (8).

For RFLP typing, the PCR product of ureC was digested

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**TABLE 1. Clinical characteristics of subjects tested in this study**

<table>
<thead>
<tr>
<th>H. pylori infection</th>
<th>No. of persons</th>
<th>Mean age (yrs) ± SE</th>
<th>% with history of:</th>
<th>% with atrophic gastritis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>77</td>
<td>58.9 ± 1.0</td>
<td>29.9</td>
<td>9</td>
</tr>
<tr>
<td>Negative</td>
<td>63</td>
<td>59.3 ± 1.3</td>
<td>3.2</td>
<td>1.6</td>
</tr>
</tbody>
</table>

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*No significant difference by Student’s t test.

*P < 0.01 by Student’s t test.

*P < 0.01 by Fisher’s exact test.
with restriction enzymes *Hha*I, *Mbo*I, and *Acc*II (Takara Shuzo Co. Ltd.) and the PCR product of *ureB* was digested with *Hae*III (Boehringer GmbH, Mannheim, Germany). These reactions were performed for 18 h at 37°C in buffer recommended by the supplier.

The digested products were analyzed by electrophoresis using a 4% agarose gel for *ureC* and a 3% agarose gel for *ureB*. *Hinc*II-digested φX174 (Toyobo Co. Ltd., Osaka, Japan) was used as the standard for molecular size determinations. The gel was stained with ethidium bromide and then examined with UV transillumination and photographed.

Seventy-seven of 140 people (70 couples; mean age 58.9 ± 1.0 years) were infected with *H. pylori*, and 63 of the 140 (mean age, 59.3 ± 1.3 years) were not. There was no significant difference in mean age between these two groups.

Of the *H. pylori*-positive subjects, 29.9% had a history of peptic ulceration, compared with only 3.2% of the *H. pylori*-negative people. Of the *H. pylori*-positive subjects, 71.4% had atrophic gastritis, but in contrast, 44.4% of the *H. pylori*-negative people had atrophic gastritis. There were significant differences in the incidence of a history of peptic ulcer and the finding of atrophic gastritis between the two groups (Table 1).

RFLP patterns of *H. pylori* were then investigated to determine whether interspousal transmission did, in fact, occur. *H. pylori* was isolated from both partners in 21 couples (group 1).
In 35 of 70 couples (group 2), *H. pylori* was isolated from one partner only. We analyzed the RFLP patterns of the *ureB* and *ureC* genes of the isolates for both partners in group 1.

Twenty of the 21 couples had different patterns, and both partners of only 1 couple showed the same *ureB* and *ureC* digestion patterns (Fig. 1). Parente et al. (10) also reported that having an *H. pylori*-positive partner with a duodenal ulcer may increase the risk of *H. pylori* colonization. However, they checked only the serum titer of immunoglobulin G anti *H. pylori* antibody and did not identify the strains of *H. pylori*. Georgopoulos et al. (4) reported that 56% of the partners of *H. pylori*-positive patients with a duodenal ulcer harbored the same strains of *H. pylori* as their spouses, as determined by the ribotyping method with 16S rRNA, the conserved part of its genes. There are several possible reasons for the difference between the methods used to identify *H. pylori* strains. Our method appears to be superior in differentiating between the strains of *H. pylori* (1, 3, 9), since the *ureB* and *ureC* genes are variable. Secondly, it may originate from cultural differences between Japan and other countries, for example, differences in behavior involving intimate contact between spouses in Japan and Western countries, such as intimate kissing. Thirdly, the divergence of *H. pylori* strains between spouses might be due to spontaneous rearrangements or in vivo selection of mutant strains after interspousal transmission of *H. pylori*. This seems unlikely, since the persons with *H. pylori* enrolled in this study always revealed the same pattern of *H. pylori* during the follow-up period.

In conclusion, we concluded that in Japan, interspousal transmission of *H. pylori* occurs rarely, even though *H. pylori* seems to be transmitted perorally (5).

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REFERENCES