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Helicobacter pylori May Be Transmitted through Gastrofiberscope Even after Manual Hyamine Washing

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Endoscopy is an effective diagnostic technique for gastric cancer, which is believed to be associated with *Helicobacter pylori*. Manual Hyamine washing is a widely used fiberscope cleaning method. Urease B gene of *Helicobacter pylori* was detected in 50% of the wash-out samples from the biopsy-suction channel of a fiberscope after manual Hyamine washing by nested polymerase chain reaction, and bacterial culture revealed viable *Helicobacter pylori* in 19%. However, *Helicobacter pylori* was not detected by either of the above methods in the biopsy-suction channel of the fiberscope after mechanical washing. These findings indicate that manual Hyamine washing of fiberscopes is insufficient to prevent iatrogenic *Helicobacter pylori* transmission, and that mechanical washing after manual Hyamine washing is essential.

Key words: *Helicobacter pylori* — Transmission — Endoscopy

Gastric cancer is one of the most common malignancies in the world.[1)] Endoscopy is an effective diagnostic technique for gastric cancer, and its use is becoming widespread. Several patients are usually examined in the same day by a single fiberscopic instrument with manual Hyamine (benzethonium chloride) washing, when the patients are all negative for spirochetes, hepatitis B virus, hepatitis C virus and human immunodeficiency virus. This is a routine procedure not only in our hospital but also in most hospitals in Japan and other countries in Asia, Africa, East Europe and South America, although there might be some variations.

Infection with *Helicobacter pylori* (*H. pylori*[6]) has been claimed to be linked with chronic gastritis, peptic ulcer and gastric cancer.[2–5)] Although the mode of *H. pylori* transmission is not established,[6)] *H. pylori* may be transmitted iatrogenically through endoscopy, because some cases of postendoscopic acute gastric mucosal lesion have been reported to be associated with *H. pylori* infection.[7,8)] Here we report that *H. pylori* was detected in 50% of the wash-out samples from the biopsy-suction channel of a fiberscope after manual Hyamine washing by polymerase chain reaction (PCR) and that bacterial culture revealed viable *H. pylori* in 19% of the wash-out samples.

The essential point of fiberscopic sterilization lies in cleaning the outer sheath as well as the biopsy-suction channel, through which biopsied samples are collected and gastric mucus is sucked. The routine procedure for washing the fiberscope between examination is as follows: the outer sheath of the fiberscope after use is wiped with a Hyamine-soaked paper towel, a 0.2% Hyamine solution is sucked through the biopsy-suction channel, 40 ml of 0.2% Hyamine solution is poured into the biopsy-suction channel, the fiberscope is rinsed with water, the fiberscope is wiped with a Hyamine-soaked paper towel again, and then the fiberscope is used for the next patient. After the examination of the last patient of the day, the fiberscope is washed with Hyamine, the washed in an automatic washing machine (Olympus EW-20) with Tego 51 (alkyldiaminoethylglycine hydrochloride).

To detect *H. pylori* attached to the biopsy-suction channel of the fiberscope, 40 ml of 0.9% NaCl solution was poured into the biopsy-suction channel of the fiberscope after the examination of the last patient of the day and after the routine washing with Hyamine, and the 0.9% NaCl wash solution was collected. The fiberscope was then washed in an automatic washing machine, followed by washing of the biopsy-suction channel with 40 ml of 0.9% NaCl solution, and the 0.9% NaCl wash solution was again collected. The 0.9% NaCl solutions were each concentrated to 1 ml by aspiration after centrifugation, and were analyzed by PCR and bacterial culture.

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[6] Abbreviations: *H. pylori*, *Helicobacter pylori*; PCR, polymerase chain reaction.

PCR analyses were done with oligonucleotide primers specific to a portion of the published sequence of urease B gene of *H. pylori*.[9, 10] The DNA sequences of primers were as follows: primer P1 (sense), 5′-GCAAGCGGTGTAACAACCAT-3′; primer P2 (sense), 5′-AAGGTAACGCTTCTAACGAT-3′; primer P3 (antisense), 5′-TGGCTTAACGCAATGAATCC-3′; primer P4 (antisense), 5′-AAGTGATGTTTGCATCGTAT-3′. PCR was performed in 50 m*M* KCl, 10 m*M* Tris-HCl (pH 9.0), 1.5 m*M* $MgCl_2$, 200 μM each of the dNTPs and 0.1% w/v Triton X-100, in a volume of 100 μl containing 10 μl of the samples, 50 pmol of each primer, 1 unit of Perfect Match (Stratagene, USA), and 2.5 units of *Taq* DNA polymerase. Concentrated wash solutions were heat-denatured at 94°C for 10 min, and then were used for the first-round PCR with primers P1 and P4, consisting of 30 cycles of 1 min denaturation at 94°C, 2 min annealing at 60°C, and 3 min extension at 72°C. To improve the sensitivity and specificity of detection, 4% of the product was subjected to a second round of 30 cycles with the internal primers P2 and P3 (nested PCR). One-tenth volume of the final reaction mixture was subjected to electrophoresis on 3.0% agarose gel and visualized by ethidium bromide staining.

The same concentrated wash solutions were also inoculated on Columbia agar base, supplemented with 7% horse serum, vancomycin (10 mg/liter), cephthrodin (5 mg/liter), trimethoprim (5 mg/liter) and amphotericin B (5 mg/liter). Plates were incubated at 37°C under microaerobic conditions in an anaerobic jar with a carbon dioxide generator envelope (BBL 70304). *H. pylori* was identified as Gram-negative bacteria, which were positive for oxidase, catalase and urease activities.[11]

We examined the fiberscope after the examination of the last patient of the day and after the routine washing with Hyamine. To our surprise, urease B gene of *H. pylori* was detected in 50% (8/16) of concentrated wash-out solutions from the biopsy-suction channel of the fiberscope after manual Hyamine washing, but not after mechanical washing, by nested PCR (Fig. 1 and Table I).

To demonstrate the presence of viable *H. pylori* in the biopsy-suction channel of the fiberscope, bacteriological analyses were performed by using the same sample examined by nested PCR. Bacteriological analyses showed the presence of *H. pylori* in 19% (3/16) after manual Hyamine washing, but in none after mechanical washing (Table I).

Our studies have shown the existence of viable *H. pylori* in the biopsy-suction channel of a fiberscope even after manual Hyamine washing, but not after mechanical washing. *H. pylori* resides in the mucus layer of the stomach, although a fraction of the bacterial cells directly adhere to the gastric epithelium.[2, 12] Gastric mucus

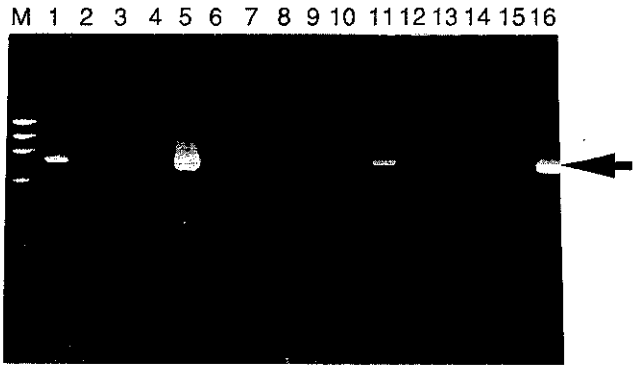

Fig. 1. Detection of urease B gene sequence of *H. pylori* by nested PCR. Lane M, ϕX174 DNA digested with *Hae*III (marker); lanes 1, 3, 5, 7, 9, 11, 13, after manual Hyamine washing; lanes 2, 4, 6, 8, 10, 12, 14, after mechanical washing (lanes 1-2, 3-4, 5-6, 7-8, 9-10, 11-12, 13-14 from the same fiberscope); lane 15, negative control (water); lane 16, positive control (ATCC *H. pylori* 43629). The arrow indicates urease B gene product (784 bp).

Table I. Detection of *Helicobacter pylori*

	Helicobacter pylori-positive fiberscope wash-out/ No. examined	
	After manual washing	After mechanical washing
PCR	8/16 (50%)	0/16 (0%)
Culture	3/16 (19%)	0/16 (0%)

containing viable *H. pylori* is sucked through the biopsy-suction channel of the fiberscope, and adheres to the wall of the channel. Manual Hyamine washing of the fiberscope is not sufficient to wash out the gastric mucus. Viable *H. pylori* attached to the wall of the biopsy-suction channel may be inoculated into another patient by the insertion of clean biopsy forceps or by the pouring of clean water. Thus, our results suggested that *H. pylori* may be transmitted iatrogenically through endoscopy, even if the instrument is cleaned by manual Hyamine washing.

Recently, three cases of endoscopic cross-infection with *H. pylori* have been demonstrated by restriction endonuclease analysis of bacterial genomic DNA.[7] It has also been reported that 10 of 19 *H. pylori*-negative patients showed immunoglobulin G seroconversion to *H. pylori* after the onset of postendoscopic acute gastric mucosal lesion.[8] Inoculated *H. pylori* required several days of incubation to produce symptoms, because the symptoms of postendoscopic acute gastric mucosal lesion

appeared several days after endoscopy.[13] These results, coupled with our results, strongly suggest that post-endoscopic acute gastric mucosal lesion is an iatrogenic disease caused by *H. pylori* infection.

Recent reports on intrafamilial transmission of *H. pylori* suggested person-to-person transmission.[14] *H. pylori* may be transmitted through saliva as well as through endoscopy, because genomic DNA sequences corresponding to species-specific antigen of *H. pylori*[15] have been reported to be amplified not only from gastric juice, but also from salive.[16] We are presently performing PCR to detect urease B gene of *H. pylori* from saliva of Japanese patients.

In conclusion, manual Hyamine washing of a fiberscope is an inadequate cleaning method, and it is most important to wash the fiberscope in an automatic washing machine for the prevention of iatrogenic *H. pylori* transmission through endoscopy.

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