Importance of the Fiberoptic Endoscope Cleaning Procedure for Detection of Helicobacter pylori in Gastric Biopsy Specimens by PCR

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Received 17 September 1993/Returned for modification 9 November 1993/Accepted 21 December 1993

A 16S ribosomal DNA-based PCR appeared to be a sensitive test for the detection of infection by Helicobacter pylori in 31 patients when compared with culturing and histological and serological techniques. For this purpose, PCR was the only test with a positive result. H. pylori DNA was also found in gastrointestinal equipment even after standard intensive combined manual and machine cleaning. We therefore conclude that a reliable validation of PCR for the detection of H. pylori in gastric biopsy specimens is possible only when the cleaning and disinfection method used has been proven to remove all H. pylori DNA from gastrointestinal equipment. An adequate cleaning and disinfection method for the removal of H. pylori DNA from fiberoptic endoscopes is described.

At present, several PCR tests are available for the detection of microorganisms, including Helicobacter pylori (1–6, 9, 18), in clinical samples. The PCR may be an alternative diagnostic method to the rather fastidious and relatively slow culturing procedure for the detection of H. pylori in gastric tissue specimens. Several investigators found the H. pylori PCR to be more sensitive than culturing and other routine laboratory tests (1, 4, 6). However, some caution with respect to the interpretation of their data seems to be indicated, as positive PCR results have been found even in the absence of histological signs of inflammation (1). Comparable results were obtained in this study during a further validation of the clinical usefulness of the H. pylori PCR by comparing a PCR which amplifies part of the 16S RNA gene (5) with culturing and histological (Giemsa staining) and serological techniques for the detection of H. pylori infection in patients with upper abdominal complaints. Therefore, we examined whether the finding of extra H. pylori-positive gastric biopsy specimens by PCR might be attributed to the presence of amplifiable DNA in endoscopes despite standard intensive cleaning and disinfection.

Thirty-one consecutive patients from an upper gastrointestinal tract endoscope program were included in this study. Endoscopy was performed with Olympus GIFQ10, Q20, 1T10, and 1T100 endoscopes. Gastric biopsy specimens were taken with standard biopsy forceps. Two biopsy specimens from the antrum (within 2 cm of the pylorus) and five specimens from the corpus were immediately fixed in a 10% formalin solution for histological examination. A third specimen from the antrum was used for both culturing and PCR.

After each endoscopic procedure, the endoscopes were cleaned and disinfected by means of combined manual and machine cleaning and disinfection. First, the endoscopes were manually washed and the biopsy channel was flushed with an enzyme-containing soap solution; additional cleaning of the outside and the working channel with a brush was then done. Thereafter, machine cleaning was performed with one of the two systems in use in the Department of Gastroenterology at Free University Hospital. System I consists of an all-channel two-step procedure that takes approximately 10 min. This procedure involves flushing with 2% buffered glutaraldehyde for 5 min and then rinsing with 2 liters of sterile water. This system has been in use at Free University Hospital for many years. During routine weekly checks, bacterial contamination has rarely been found in the last few years (13). The other system (system II; Wassenburg, Dooodeward, The Netherlands) also consists of an all-channel procedure but is more extensive and takes approximately 30 min. It starts with a perfusion test to detect obstruction by a mucous plug that might prevent channels from being cleaned. Thereafter, all channels are flushed for 6 min with an enzymatic soap solution. Then, 2% glutaraldehyde is flushed through for the next 10 min, after which the endoscope is finally rinsed for 10 min with approximately 25 liters of sterile water. This machine has been at our disposal for 1 year, and no bacterial contamination has been found during this period. In both systems, the endoscopes are fully submerged during the cleaning. System I was used in the portion of the study concerning the collection of gastric biopsy specimens for comparison of the different diagnostic tests. Endoscopes were also checked for the presence of amplifiable DNA after cleaning and disinfection. For this portion of the study, the working channels of the above-mentioned standard endoscopes were flushed with 0.5 ml of distilled water, which was then assayed by PCR. The endoscopes were tested in two different series. During the first series, consisting of 23 test experiments, the endoscopes were mechanically disinfected by machine with system I, whereas in the second series, consisting of 21 experiments, system II was used. In both series, no H. pylori organisms were cultured from the flushing fluid samples.

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A histological examination was performed on 4-μm Giemsa-stained sections. All slides were evaluated by the same pathologist.

For culturing, biopsy specimens were transported on ice to the laboratory in brucella broth (Oxoid Ltd., Basingstoke, United Kingdom) with 5% saponin-lysed horse blood. Within 4 h, the biopsy specimens were cultured on Belo Horizonte Agar (15) by rubbing the specimen several times over the agar surface. The remaining part of the specimen was placed in lysis buffer (20 mM Tris-HCl [pH 8], 0.5% Tween 20) and frozen at −20°C for PCR. Agar plates were incubated in a humid atmosphere under microaerophilic conditions. After 5 days, plates were checked for the presence of H. pylori-like gold-colored colonies. H. pylori was further identified by testing for the production of urease, oxidase, and catalase by routine bacteriological techniques.

The presence of H. pylori-specific antibodies of the immunoglobulin G class was determined by means of an enzyme-linked immunosorbent assay as described before (8, 14).

For pretreatment of samples for PCR, biopsy specimens frozen in lysis buffer were thawed and proteinase K was added to a final concentration of 0.5 mg/ml. After incubation at 55°C for 60 min, the samples were boiled for 10 min and cooled on ice immediately afterwards. Of these pretreated samples, 10 μl was used for PCR. Endoscope flushing fluids were supplemented with 20 mM Tris-HCl (pH 8)-0.5% Tween 20-0.5 mg of proteinase K per ml and processed as described above. For PCR, primers directed to the 16S rRNA gene as described by Ho et al. (5) were used and resulted in the amplification of a 109-bp DNA fragment. The nucleotide sequences of the primers were as follows: sense primer, Hpr1, 5′-CTGGGAG AGACTAAGCCCTC-3′; and antisense primer, Hpr2, 5′-ATTACTGACGCTGAT1GTGC-3′. Oligonucleotides were synthesized by the phosphoramidite method on a Gene-Assembler Plus (Pharmacia, Uppsala, Sweden) DNA synthesizer.

The PCR protocol of Ho et al. (5) was used with some minor modifications. In brief, the PCR was performed with a 50-μl reaction mixture containing 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 1.5 mM MgCl2, 200 μM each of the four deoxynucleoside triphosphates, 1.0 U of Taq polymerase (Amplitaq; Cetus), 50 pMol of each primer, and 10 μl of sample. The mixture was incubated for 4 min at 94°C; incubation was followed by 40 cycles of 1 min of denaturation at 94°C, 1 min of annealing at 60°C, and 1.5 min of elongation at 72°C, each in a PCR processor (Biomed 60; Theres, Germany). After the last cycle, a temperature delay step of 4 min at 72°C was included to complete chain elongation. To check for inhibitory substances in the processed biopsy specimens, we performed a separate PCR directed at the human β-globin gene (17).

PCR products were analyzed by electrophoresis on a 1.5% agarose gel and ethidium bromide staining. Then, DNA was transferred from the agarose gel to a nylon membrane (Gene-Screen Plus; DuPont) by diffusion blotting in 0.4 N NaOH overnight. The PCR products were analyzed by specificity by hybridization at 55°C with a 32P-end-labelled target-specific internal oligonucleotide probe, pHpr1 (5′-CATCCATCGTTTAGGGCGTG-3′) (5), and autoradiography according to standard procedures (11).

By PCR, 1 to 10 CFU was detected after hybridization of a dilution series of four clinical isolates of H. pylori with an internal specific oligonucleotide probe (data not shown).

Endoscopy of 31 patients revealed discrete signs of gastritis in 10 patients, peptic esophagitis in 1 patient, and no mucosal abnormalities in 20 patients. No further abnormalities were noted in any patient. Eleven of the 31 patients (35%) were considered truly positive for H. pylori, because at least three diagnostic tests showed positive results, with the following scores: PCR, 11 of 11 (100%); culturing, 8 of 11 (73%); Giemsa staining, 10 of 11 (91%); and serological testing, 11 of 11 (100%). Of the 11 PCR-positive samples, 10 were visible after both gel analysis and hybridization 1 was visible only after hybridization. Typical PCR results obtained for biopsy samples are shown in Fig. 1. The smaller fragments observed are primer dimers. Fourteen patients (45%) were considered truly negative for H. pylori, as all four diagnostic tests showed negative results. No inhibition of amplification by PCR was observed in any of the biopsy specimens, as indicated by the generation of the 110-bp specific β-globin fragment at the gel level. The result of the remaining six patients were considered inconclusive. One patient (3%) was found positive only by serological testing. This result could not be explained by recent eradication therapy. Five patients (16%) were found weakly positive by PCR, as shown by the detection of H. pylori-specific amplification products only after hybridization. However, these patients were found negative by culturing and histological and serological testing. In addition, no signs of inflammation of the antrum were observed in any of these patients. Negative cultures could not have been due to sampling error, as both PCR and culturing were performed on the same biopsy specimens. These extra positive results found by PCR may have been due to a higher sensitivity, contamination at the laboratory, or contamination from endoscopes. Can false positivity be ruled out?

Contamination at our laboratory is prevented by several measures. One room is reserved for reagent stock preparation. Another room is used for PCR buffer preparation and processing of clinical specimens, each with separate pipettes. In addition, clinical material is pipetted with the use of filter tips inside a laminar flow cabinet. Finally, gel analysis of PCR products and autoradiography are performed in separate rooms. Furthermore, in each room, separate laboratory clothes and materials for administration, such as pencils, are used. In addition, we validated our PCR procedure by testing 10 gastric biopsy specimens known to be strongly positive (visible at the gel level) and 10 PCR buffer solutions according to our
standard procedure in a blinded way. This experiment was repeated twice. In all instances, the *H. pylori*-containing gastric specimens appeared to be positive by PCR, whereas in none of the buffer solutions could *H. pylori*-specific amplification products be detected by hybridization. These results indicate that our procedure for testing gastric biopsy specimens by PCR for the presence of *H. pylori* DNA is reliable. Positive PCR results due to sample contamination at the laboratory in this study are also highly unlikely, since negative controls consisting of PCR buffer to which 10 µl of twice-distilled water had been added by means of the same pipette as that used for the processing of clinical samples showed no detectable signal after amplification and analysis by hybridization.

On the other hand, gastric biopsy specimens may have been contaminated with *H. pylori* DNA remaining behind in the biopsy channels of the fiberoptic endoscopes. Indeed, it has been reported that patients can be infected with *H. pylori* through gastrointestinal equipment which is not properly disinfected (10). This report emphasizes the need to check endoscopes for the presence of culturable bacteria after cleaning and disinfection. Regular disinfection checks for gastrointestinal equipment at Free University Hospital almost never yield positive bacterial cultures (13). However, with the introduction of a new, highly sensitive diagnostic test based on the detection of bacterial nucleic acids, such as PCR, it becomes important to check routine cleaning and disinfection procedures for endoscopes, not only for disinfection, as is routinely done by us, but also for the removal of *H. pylori* DNA. Otherwise, the possibility of falsely assigning a patient positive for *H. pylori* on the basis of PCR testing cannot be ruled out.

Endoscopes are often cleaned and disinfected only manually in between the collection of gastric tissue specimens (7). In the Department of Gastroenterology at Free University Hospital, endoscopes have in addition been washed for many years by machine (system I) as described earlier. This system was additionally tested for the removal of *H. pylori* DNA from endoscopes. In 8 of 23 cases, an *H. pylori*-specific 109-bp fragment was amplified, as detected by hybridization, from the distilled water flushed through the working channels of the endoscopes after cleaning and disinfection (16). In five of these eight cases, this persistent contamination could be linked to previously examined patients who were shown to be *H. pylori* positive. In one case, a gastric tissue sample taken during the previous endoscopy was negative. However, the same endoscope proved to be *H. pylori* positive by PCR on the previous occasion. In the remaining two cases, no result for a preceding biopsy specimen was available. Of the 15 *H. pylori* PCR-negative flushing fluids, 8 were preceded by collection of an *H. pylori*-negative biopsy specimen; for the other seven, no culture or PCR result for a preceding biopsy specimen was available. An example of PCR-positive results obtained after amplification of samples collected from the working channels of endoscopes is shown in Fig. 2. In contrast, when endoscopes were cleaned and disinfected between each endoscopic procedure by a commercial automated washing system (system II), none of the 21 flushing fluids tested were found positive by *H. pylori* PCR, despite the fact that in 6 cases, an *H. pylori*-positive biopsy specimen had been collected previously. In eight cases, the preceding biopsy specimen was *H. pylori* negative, and in the remaining seven cases, no result for a preceding biopsy specimen was available. The effective removal of all *H. pylori* DNA by system II is likely to be due to the inclusion of a step for the enzymatic digestion of mucus and tissue parts and the extensive rinsing at the end of the procedure. In another study, Katoh et al. (7) showed that after machine washing and disinfection, no *H. pylori* DNA could be detected in the working channels of 16 endoscopes, in contrast to the finding of endoscopes positive for *H. pylori* by PCR (8 of 16) or even by culturing (3 of 16) after manual washing. However, the PCR-negative results obtained after machine washing theoretically could have been due to the absence of *H. pylori* in the gastric biopsy specimens in that particular series, for which no data were shown. An additional argument for our hypothesis is found in the study of Mapstone et al. (12), who demonstrated recently that gastric tissue specimens can also be contaminated with *H. pylori* DNA through cleaned and disinfected biopsy forceps.

In summary, PCR appears to be slightly more sensitive than culturing and at least as sensitive as serological testing and Giemsa staining for the diagnosis of *H. pylori* infections. In addition, we demonstrated that *H. pylori* DNA in fiberoptic endoscopes can be detected by PCR, depending on the method used for cleaning and disinfection. Therefore, we conclude that before PCR can be evaluated with respect to the detection of *H. pylori* DNA in gastric tissues, methods for the cleaning and disinfection of endoscopic equipment must be evaluated for the removal of *H. pylori* DNA. The latter can be achieved by extensive washing procedures, as shown by our data.

We thank Rick Piqué and Gerrie Hop for expert technical assistance.

REFERENCES


