Eradication of *Helicobacter pylori* According to 23S Ribosomal RNA Point Mutations Associated With Clarithromycin Resistance

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**Background.** Clarithromycin-resistant *Helicobacter pylori* is associated with point mutations in the 23S ribosomal RNA (rRNA) gene.

**Methods.** A total of 1232 patients participated and were divided into 2 control groups and 1 case group. Patients in the APC control group, which consisted of 308 randomly assigned participants, were treated with standard triple therapy, consisting of amoxicillin, rabeprazole, and clarithromycin; 308 participants in the APM control group were treated with amoxicillin, rabeprazole, and metronidazole. For the 616 participants in the case group, a test for point mutations in the 23S rRNA gene of *H. pylori* was conducted. A total of 218 individuals in the case group received a new tailored therapy regimen, in which amoxicillin, rabeprazole, and clarithromycin were given in the absence of a mutation, whereas clarithromycin was replaced by metronidazole if the mutation was detected.

**Results.** The rate of eradication of *H. pylori* in the tailored group was 91.2% (176/193), which was significantly higher than that in the APC (75.9% [214/282]; *P* < .001) and APM (79.1% [219/277]; *P* < .001) control groups.

**Conclusion.** The rate of *H. pylori* eradication among patients who received tailored therapy on the basis of detection of a clarithromycin resistance mutation by polymerase chain reaction was much higher than the rate among patients who received a standard triple therapy regimen.

**Clinical Trials Registration.** NCT0145303.

**Keywords.** *Helicobacter pylori*; point mutation; Clarithromycin; drug resistance.

*Helicobacter pylori* may cause chronic gastritis and peptic ulcers and is associated with gastric cancer and mucosa-associated lymphoid tissue lymphoma. Thus, the eradication of *H. pylori* may inhibit the recurrence of peptic ulcers and prevent gastric cancer [1]. The Second Asia–Pacific Consensus Guidelines [2], guidelines for the management of *H. pylori* in Japan [3], and treatment guidelines in South Korea [4] recommend the administration of the proton-pump inhibitors (PPIs) clarithromycin and amoxicillin for 7 or 14 days to eradicate *H. pylori*.

With an eradication rate of >90%, the standard triple therapy was notably effective until 1998 [5]; however, the rate decreased to <80% after 2000 because of increasing antibiotic resistance, especially to clarithromycin [6–8]. The rate of resistance to clarithromycin in South Korea was 5.9% before 2000 [9], but it increased to 13.8% in 2003 [10] and has rapidly increased to 32.0% since 2003 [11]. Therefore, the eradication rate may be increased by selecting antibiotics based on clarithromycin resistance. *H. pylori* culture and antibiotic resistance testing are necessary to determine drug resistance, but clinically there are many limitations to these methods, including the difficult and lengthy process of bacterial *H. pylori* cultivation.

Clarithromycin resistance against *H. pylori* is associated with point mutations in the 23S ribosomal RNA (rRNA) gene. When a point mutation occurs, the binding of clarithromycin to the ribosome decreases, causing resistance [12]. The point mutations can be identified using dual-priming oligonucleotide (DPO)–based multiplex
polymerase chain reaction (PCR). There are advantages in using DPO-based multiplex PCR, including the availability of results within a short period and the possibility of detection even when the mutation accounts for only 2% of the total 23S rRNA genes [13].

We introduced a new method of tailored therapy in this field, in which patients were selectively treated with appropriate medications according to the result of a pretreatment test. The existing standard triple therapy including clarithromycin was used for the absence of a point mutation; otherwise, the strain was considered clarithromycin-resistant and was treated with metronidazole instead of clarithromycin in the triple-drug regimen. The outcome of tailored therapy was compared to that of the existing standard triple therapy, which was given without consideration of the presence of a point mutation. We hypothesized that the rate of H. pylori eradication may be improve if different antibiotics are used, depending on the presence of clarithromycin resistance.

MATERIALS AND METHODS

Subjects

Patients from 5 Catholic University of Korea-affiliated hospitals (Yeouido St. Mary’s Hospital, Bucheon St. Mary’s Hospital, Incheon St. Mary’s Hospital, St. Vincent’s Hospital, and St. Paul’s Hospital) who had endoscopically confirmed gastric or duodenal ulcer were recruited from July 2011 to March 2012. The subjects were aged 20–75 years. Patients were excluded if they had received H. pylori eradication therapy; had a history of gastric surgery, malignant tumors, and/or systemic disorders, such as severe organ dysfunction; and/or were pregnant.

Study Design

A total of 1232 patients participated and were divided into 2 control groups and 1 case group. The sample size of 1232 patients was calculated as follows. To achieve a significance level of 0.05 and 90% statistical power, 262 patients were needed in each group, assuming eradication rates of 90% in the case group and 80% in the control group. Six hundred sixteen patients were therefore required for the case group, assuming a dropout rate of 15%.

The 2 control groups consisted of 616 patients with H. pylori infections confirmed by detection of the Warthin-Starry stain in an endoscopically obtained biopsy specimen. Patients were randomly divided into one of 2 groups by use of a random number table and received treatment for 7 days to eradicate H. pylori. A total of 308 patients were treated with amoxicillin, rabeprazole, and clarithromycin (the APC group), while the other 308 patients were treated with amoxicillin, rabeprazole, and metronidazole (the APM group).

Warthin-Starry stain and PCR of gastric mucosa biopsy specimens was performed in 616 patients in the case group, and the diagnostic criterion standard was PCR. The A2142G and A2143G mutations associated with clarithromycin resistance were identified using DPO-based multiplex PCR. Patients with no mutation detected in H. pylori were considered to have bacteria susceptible to clarithromycin and were treated with amoxicillin, rabeprazole, and clarithromycin. Patients with H. pylori containing this resistance mutation (the tailored group) were considered to have bacteria resistant to clarithromycin and received tailored therapy consisting of amoxicillin, rabeprazole, and metronidazole. Mucosal biopsies were performed in all patients to obtain 2 tissue specimens each from the antrum and body, which were subjected to Warthin-Starry silver staining.

The tailored group underwent additional mucosal biopsies to obtain 1 specimen each from the antrum and body for PCR analysis. The tailored group was limited to patients confirmed to have H. pylori infection according to the DPO-based multiplex PCR.

For the 180 patients in the tailored group, the C13-urea breath test was performed before initiation of eradication therapy; findings of the breath test were subsequently compared with those of DPO-based multiplex PCR. The C13-urea breath test was performed in all the patients 6–8 weeks after treatment, to confirm eradication.

This study was approved by the institutional review board at the Catholic University (SC11OASI0161) and is registered on ClinicalTrials.gov (NCT01453036).

Clarithromycin Mutation Test

DNA Isolation

DNA was isolated by the QIAamp DNA mini kit (Qiagen, Valencia, CA) from frozen gastric tissue biopsy specimens that were stored at a temperature of less than −20°C. The tissue specimens were placed in a microcentrifuge tube, and buffer ATL (180 µL) and proteinase K (20 µL) were added. The samples were mixed by vortexing and incubated at 56°C until the tissues were completely lysed. Buffer AL (200 µL) was added to the samples, which were subsequently incubated at 70°C for 10 minutes. Next, 240 µL of 100% ethanol was added to the samples, which were mixed by vortexing for 15 seconds. Each sample was placed in a QIAamp spin column and centrifuged at 8000 rpm for 1 minute. The columns were washed with AW1 buffer (500 µL), and samples were centrifuged at 8000 rpm for 1 minute. AW2 buffer (500 µL) was added to the column, and samples were centrifuged at 14 000 rpm for 3 minutes. Buffer AE (200 µL) was added to each sample, and samples were incubated for 1 minute prior to centrifugation at
8000 rpm for 1 minute. Finally, the DNA was extracted from the tissue.

Gene Mutation Test: DPO-Based Multiplex PCR
Point mutation–containing gene fragments were specifically amplified using the SeeplexClara-H. pylori ACE detection system (Seegene, Seoul, Korea), which was developed with the DPO technology. After an initial incubation step at 94°C for 15 minutes, 40 amplification cycles were performed in a GeneAmp PCR system 9700 (Applied Biosystems, Foster City, CA), using the following amplification parameters: 94°C for 30 seconds, 65°C for 30 seconds, and 72°C for 1 minute. The final extension was performed at 72°C for 10 minutes.

Data Analysis
The amplified DNA products (5 µL of PCR products and 5 µL of Clara-HP marker) were identified on a UV transilluminator after electrophoresis using a 2% agarose gel containing ethidium bromide. The presence of a single 621-bp product in the amplified DNA was construed as belonging to wild-type H. pylori. DNA bands at 475 bp and 194 bp indicated the presence of the A2142G and A2143G mutations, respectively (Figure 1).

Eradication of H. pylori
All patients received eradication therapy for 7 days. Patients in the APC group were treated twice daily with 1000 mg of amoxicillin, 20 mg of rabeprazole, and 500 mg of clarithromycin. Patients in the APM group received 1000 mg of amoxicillin, 20 mg of rabeprazole twice daily, and 500 mg of metronidazole three times daily. In the absence of a 23S rRNA point mutation in H. pylori, the treatment for the tailored group was the same as that for the APC group, which included clarithromycin. If a mutation was present, the treatment was the same as that for the APM group, which included metronidazole.

Eradication was determined by the C14 urea breath test 6–8 weeks after completion of eradication therapy, when PPIs had not been used for at least 2 weeks. To verify compliance with the medication, the patients were asked to bring their remaining medicines, which were counted; patients who were <80% compliant were excluded from the per-protocol analysis.

Factors Affecting H. pylori Eradication and Adverse Reactions to Medicine
Gastric ulcers, duodenal ulcers, smoking, drinking, body mass index, and total cholesterol levels were examined as factors that may affect the eradication of H. pylori. After 7 days of eradication therapy, 17 potential adverse reactions were investigated in a standardized way, including the following: changes in taste, nausea, vomiting, diarrhea, headache, and skin allergies.

Statistical Analysis
The results of this study were analyzed in an intention-to-treat population and a per-protocol population. SAS, version 9.2 for Windows, was used for the statistical analyses, in which the eradication rate was analyzed by the \( \chi^2 \) test, and the factors affecting eradication were analyzed by multivariable logistic regression. \( P \) values of <.05 were deemed statistically significant.

RESULTS
Characteristics of Patients
There was no difference in the age of subjects in each group, with mean values (SD) of 54.7 ± 12.2 years for the tailored group, 54.0 ± 12.0 years for the APC group, and 56.2 ± 13.2 years for the APM group. In the tailored group, gastric ulcers were present in 57.8% of the subjects (126/218), and duodenal ulcers were observed in 42.2% (92/218). In the APC group, there was no difference in the age of subjects in each group, with mean values (SD) of 54.7 ± 12.2 years for the tailored group, 54.0 ± 12.0 years for the APC group, and 56.2 ± 13.2 years for the APM group. In the tailored group, gastric ulcers were present in 57.8% of the subjects (126/218), and duodenal ulcers were observed in 42.2% (92/218). In the APC group,

### Table 1. Characteristics of Patients, by Study Group

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Tailored Group (n = 218)</th>
<th>APC Group (n = 308)</th>
<th>APM Group (n = 308)</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>54.7 ± 12.2</td>
<td>54.0 ± 12.0</td>
<td>56.2 ± 13.2</td>
<td>.112</td>
</tr>
<tr>
<td>Male sex</td>
<td>103 (47.2)</td>
<td>169 (54.9)</td>
<td>158 (51.3)</td>
<td>.243</td>
</tr>
<tr>
<td>Gastric ulcer</td>
<td>126 (57.8)</td>
<td>169 (54.9)</td>
<td>175 (56.8)</td>
<td>.271</td>
</tr>
</tbody>
</table>

Data are no. (%) of patients or mean value ± SD. 
Abbreviations: APC, amoxicillin, rabeprazole, clarithromycin; APM, amoxicillin, rabeprazole, metronidazole.

\( a \) By 1-way analysis of variance.

\( b \) By the \( \chi^2 \) test.
54.9% (169/308) had gastric ulcers, and 45.1% (139/308) had duodenal ulcers. In the APM group, 56.8% (175/308) had gastric ulcers, and 43.2% (133/308) had duodenal ulcers. These results indicate that there were no differences among the 3 groups (Table 1).

**Rates of H. pylori Detection by DPO-based Multiplex PCR**

On the basis of the C13-urea breath test, rates of H. pylori detection by DPO-based multiplex PCR had a sensitivity of 87.5%, a specificity of 91.3%, a positive predictive value of 84.0%, a negative predictive value of 93.3%, and an accuracy of 90.0% (Table 2).

**Prevalence of 23S rRNA Point Mutations**

Of the 616 patients in the tailored group, 263 were confirmed by the DPO-based multiplex PCR test to have H. pylori infection. Thus, the rate of H. pylori infection was 42.7% (263/616). Of the 263 infected patients, H. pylori in 57 (21.7%) had 23S rRNA point mutations associated with clarithromycin resistance. The mutation subtypes included A2143G in H. pylori from 53 patients and A2142G in H. pylori from 4 patients.

**Rates of H. pylori Eradication**

The intention-to-treat analysis was conducted on 218, 308, and 308 patients in the tailored group, APC group, and APM group, respectively. The per-protocol analysis was conducted on 193, 282, and 277 patients, respectively; patients were excluded because they did not undergo the C13-urea breath test or were <80% compliant (Figure 2).

In the per-protocol analysis, eradication was achieved in 91.2% patients (176/193) in the tailored group, 75.9% (214/282) in the APC group, and 79.1% (219/277) in the APM group ($P < .001$). In the intention-to-treat analysis, eradication was achieved by 80.7% (176/218) in the tailored group, 69.5% (214/308; $P = .004$) in the APC group, and 71.1% (219/308; $P = .012$) in the APM group (Table 3).

Depending on the presence of the 23S rRNA point mutation, the rate of wild-type H. pylori eradication was 94.9% (131/138), and the rate of mutant H. pylori eradication was 81.8% (45/55) ($P = .004$).

**Factors Affecting H. pylori Eradication**

The existence of peptic ulcer, smoking, drinking, body mass index, or dyslipidemia did not have an effect on eradication (Table 4).

**Adverse Reactions**

Mild adverse reactions occurred in 26.8% of patients (112/418) in the APC group and 29.3% (98/334) in the APM group. Bitter...
taste was most common in the APC group, followed by loose stool, and nausea; nausea was most common in the APM group, followed by dyspepsia and abdominal bloating (Table 5).

DISCUSSION

To eradicate *H. pylori*, the American College of Gastroenterology recommends the administration of PPI and clarithromycin, as well as amoxicillin or metronidazole, for 14 days as a first-line therapy [14]. The Maastricht IV consensus recommends the administration of PPI and clarithromycin, as well as amoxicillin or metronidazole, for 7 days when the clarithromycin resistance is <20% and the administration of bismuth, PPI, metronidazole, and tetracycline, when the clarithromycin resistance is >20% [15]. Meanwhile, the Second Asia–Pacific Consensus Guidelines [2], the treatment guidelines in Japan [3], and the treatment guidelines in South Korea [4] all recommend the administration of PPI, clarithromycin, and amoxicillin for 7 or 14 days.

The above guidelines for the eradication of *H. pylori* include clarithromycin as a first-line therapy, which has shown high eradication rates (ie, >90%) when used as part of the triple therapy regimen. Recently, however, the resistance to clarithromycin has increased with the increasing use of macrolide antibiotics as treatment for respiratory disease, and accordingly, the eradication rate has been consistently decreasing [16]. In South Korea, where the prevalence of *H. pylori* is high, the rate of eradication with the standard triple therapy regimen was notably effective (ie, >90%) until 1998 [5], but since 2000, the eradication rate has been lower (77.0%–84.5%) [6–8]. The eradication rate in Japan also recently decreased to <80% [3]. The eradication rate of 77.0% recently observed in a per-protocol analysis of the standard triple therapy regimen [6] does not meet the requirements of the Asia–Pacific Consensus Guidelines, which stipulate that success rates of >90% and >80% must be shown in per-protocol and intention-to-treat analyses, respectively, for a regimen to be considered suitable for first-line eradication therapy [2, 4]. Consequently, there has been controversy over whether the current standard triple therapy regimen, which has an eradication rate <80%, should continue to be used.

### Table 3. Rates of *Helicobacter pylori* Eradication in the Per-Protocol and Intention-to-Treat Populations

<table>
<thead>
<tr>
<th>Population</th>
<th>Achieved/Analyzied, Patients, No.</th>
<th>Patients, % (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Per protocol</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tailored</td>
<td>176/193</td>
<td>91.2 (88.9–93.4)</td>
<td></td>
</tr>
<tr>
<td>APC control</td>
<td>214/282</td>
<td>75.9 (70.2–81.6)</td>
<td>&lt;.001a</td>
</tr>
<tr>
<td>APM control</td>
<td>219/277</td>
<td>79.1 (73.7–84.5)</td>
<td>&lt;.001a</td>
</tr>
<tr>
<td>Intention to treat</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tailored</td>
<td>176/218</td>
<td>80.7 (76.2–85.2)</td>
<td></td>
</tr>
<tr>
<td>APC control</td>
<td>214/308</td>
<td>69.5 (62.9–76.1)</td>
<td>.004a</td>
</tr>
<tr>
<td>APM control</td>
<td>219/308</td>
<td>71.1 (65.3–76.9)</td>
<td>.012a</td>
</tr>
</tbody>
</table>

Abbreviations: APC, amoxicillin, rabeprazole, clarithromycin; APM, amoxicillin, rabeprazole, metronidazole; CI, confidence interval.

* a Compared with the tailored group.

### Table 4. Factors Affecting *Helicobacter pylori* Eradication

<table>
<thead>
<tr>
<th>Factor</th>
<th>Value</th>
<th>Crude OR (95% CI)</th>
<th>P</th>
<th>Adjusted OR (95% CI)a</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diagnosis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gastric ulcer</td>
<td>369 (60.5)</td>
<td>1.045 (.721–1.514)</td>
<td>.817</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Duodenal ulcer</td>
<td>241 (39.5)</td>
<td>Reference</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alcohol use</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>167 (27.4)</td>
<td>1.114 (.725–1.712)</td>
<td>.622</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>443 (72.6)</td>
<td>Reference</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smoking status</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smoker</td>
<td>97 (15.9)</td>
<td>1.392 (.789–2.453)</td>
<td>.254</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nonsmoker</td>
<td>513 (84.1)</td>
<td>Reference</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI, mean ± SD</td>
<td>22.91 (2.81)c</td>
<td>1.042 (.975–1.115)</td>
<td>.227</td>
<td>1.022 (.940–1.111)</td>
<td>.613</td>
</tr>
<tr>
<td>Total cholesterol level (mg/dl), mean ± SD</td>
<td>561 (200.90 ± 36.20)d</td>
<td>0.994 (.988–1.001)</td>
<td>.083</td>
<td>0.996 (990–1.003)</td>
<td>.251</td>
</tr>
</tbody>
</table>

Values are no. (%) of patients, unless otherwise indicated.

Abbreviations: CI, confidence interval; OR, odd ratio.

* a All variables that showed at least a weak association (ie, a P value of ≤.25) with the success of eradication were adjusted for during multivariable logistic regression.

b Body mass index (BMI) is calculated as the weight in kilograms divided by the height in meters squared.

c Data are missing for 31 subjects.

d Data are missing for 49 subjects.
In South Korea, the antibiotic resistance rates for amoxicillin, metronidazole, and clarithromycin were 0%, 40.6%, and 5.9%, respectively, before 2000 [9]. However, these rates increased to 18.5%, 66.2%, and 13.8%, respectively, in 2003 [10]. Between 2003 and 2009, the resistance rates to amoxicillin and metronidazole decreased to 4.5% and 29.7%, respectively, but the resistance rate to clarithromycin increased drastically, to 32.0% [11]. The mechanism for clarithromycin resistance in *H. pylori* is associated with 23S rRNA point mutations, which typically involve the base substitution from adenine to guanine at base positions 2142 or 2143 and, in rare cases, involve the base substitution of adenine to cytosine at position 2142 [17, 18]. The proportion of patients with the A2143G mutation was found to be 93%, which accounted for the vast majority of patients with a point mutation. These point mutations cause clarithromycin resistance because the binding of clarithromycin to ribosome is reduced [12]. When there is resistance to clarithromycin, an all or nothing phenomenon occurs in which the effects of treatment fall significantly even if the clarithromycin dosage is increased. Thus, to increase the eradication rate, it is important to selectively administer medicines by first determining clarithromycin resistance [19, 20]. However, there are many limitations in using bacterial culture and susceptibility testing to identify resistance in a clinical practice before initiation of actual eradication therapy because it is difficult to culture *H. pylori* and because it takes approximately 10 days to isolate and culture *H. pylori* and measure the minimal inhibitory concentration. Meanwhile, DPO-based multiplex PCR has certain advantages over culture and susceptibility testing, including a short turnaround time for pathogen identification and mutation detection. In addition, clarithromycin resistance in *H. pylori*, as well as the presence of *H. pylori*, can be identified through the detection of 23S rRNA point mutations. The presence of *H. pylori* can be verified if the PCR products are only observed at 621 bp, using electrophoresis. The A2143G mutation is present if a 475-bp band is also observed, whereas the A2142G mutation is present if a 194-bp band is present. The specificity of the DPO-based multiplex PCR is higher than that of the existing PCR method because the existing PCR method can produce nonspecific PCR products. DPO-based multiplex PCR, however, does not produce nonspecific PCR products because it uses 2 primers, with different lengths, that are connected to the polydeoxyinosine linker, allowing higher specificity than the existing PCR method. In other words, the longer 5′ region stabilizes the annealing process by easily binding to the template DNA, and the shorter 3′ region blocks nonspecific annealing by readily binding to the base sequence [21].

Of the 263 patients with *H. pylori* infection, *H. pylori* in 57 had point mutations in the 23S rRNA gene associated with clarithromycin resistance; A2143G was found in *H. pylori* from
In this study, the clarithromycin resistance rate was 21.7% by the DPO-based multiplex PCR. Thus, selective treatment based on findings of the DPO-based multiplex PCR test, which can confirm *H. pylori* infection and clarithromycin resistance, may prevent exposure to unnecessary antibiotics and increase the eradication rate. In conclusion, the eradication rate can be considerably improved by introducing clarithromycin resistance testing before initiation of eradication therapy, using the fast and inexpensive DPO-based multiplex PCR, and by subsequently selecting the appropriate medication regimen.

**Notes**

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**References**