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IMPROVEMENTS IN *HELICOBACTER PYLORI* ERADICATION RATES THROUGH CLINICAL *CYP2C19* GENOTYPING

TAKASHI TAMURA[1], MIO KURATA[1], SHIGERU INOUE[2], TAKAAKI KONDO[3], YASUYUKI GOTO[1], YOSHIKAZU KAMIYA[4], SAYO KAWAI[1] and NOBUYUKI HAMAJIMA[1]

[1]*Department of Preventive Medicine, Nagoya University Graduate School of Medicine*
[2]*Medical Student, Nagoya University School of Medicine*
[3]*Nagoya University School of Health Sciences*
[4]*Department of Hematology and Oncology, National Hospital Organization, Higashi Nagoya National Hospital*

ABSTRACT

Lansoprazole (LPZ), amoxicillin (AMPC) and clarithromycin (CAM) are commonly used drugs (LAC regimen) for *Helicobacter pylori* (*H. pylori*) eradication, but the eradication rate with this regimen was reported to be 70% to 90%. A few studies have reported that a successful eradication was associated with the *CYP2C19* genotype, which influences the metabolism of proton pump inhibitors (PPI) including LPZ. This study examined the changes in the *H. pylori* eradication rates between the periods before and after the commencement of a routine genetic test for *CYP2C19* at the Daiko Medical Center in Nagoya, Japan, in November, 2005. Subjects were patients who visited the Center during the period from June, 2004 to August, 2010. The patients were classified into three groups according to their *CYP2C19* genotype: rapid metabolizers (RM) with a **1*1* genotype, intermediate metabolizers (IM) with a **1*2* or **1*3* genotype, and poor metabolizers (PM) with a **2*2*, **2*3*, or **3*3* genotype. Non-rapid metabolizers (IM and PM) were basically treated with a LAC regimen, while RMs were treated with a RAM regimen (rabeprazole, AMPC, and metronidazole). The eradication rate was 80.0% (n=90) for the period without the genetic testing and 88.7% (n=124) for the period with the genetic testing (χ^2=3.11, p=0.078). The age-sex adjusted odds ratio of eradication success was 2.29 (95% confidence interval, 0.99–5.28, p=0.051) for the latter period relative to the former period among those less than 70 years of age. Those results suggested that the routine genetic test which allows a choice of the RAM regimen for RM improved the eradication rate.

Key Words: *Helicobacter pylori*, *CYP2C19* genotype, Proton pump inhibitor, Eradication rate

INTRODUCTION

In Japan, a *Helicobacter pylori* (*H. pylori*) eradication therapy was approved as a treatment for gastric and duodenal ulcers in November 2000. At that time, the only approved regimen covered by health insurance was a proton pump inhibitor (PPI) + clarithromycin (CAM) + amoxicillin (AMPC). Although the reported eradication rates of this regimen were around 90% in 2000,[1)] the

Corresponding author: Takashi Tamura
Department of Preventive Medicine, Nagoya University Graduate School of Medicine,
65 Tsurumai-cho, Showa-ku, Nagoya 466-8550, Japan
Phone: +81-(0)52-744-2145, Fax: +81(0)52-744-2161
E-mail address: tamura.takashi@c.mbox.nagoya-u.ac.jp

rate has subsequently been declining to 80%.[2] One recent study reported that the eradication rate had even fallen below 80%.[3] Possible causes of such an eradication failure were CAM-resistance and genotypes influencing PPI metabolism, as well as smoking and low rates of compliance.

The major role of PPI is to enhance antibiotic effects through a pH rise in the stomach. *CYP2C19* is an enzyme metabolizing PPI whose genotypes have been classified into three groups in terms of their enzyme activity: rapid metabolizers (RM), intermediate metabolizers (IM), and poor metabolizers (PM) at a frequency of 35%, 49%, and 16% in Japan, respectively.[4] Since PPI is inactivated faster among RM, their duration of acid secretion inhibition is shorter. Although some studies reported that the difference in PPI of the first-line therapy (PPI + CAM + AMPC) did not influence the overall eradication rate,[5-7] other studies indicated that the lansoprazole (LPZ) +AMPC + CAM (LAC regimen) was less effective for RM than for IM and PM.[8,9] Accordingly, in November 2005 we started *CYP2C19* genotyping for individuals seeking *H. pylori* eradication therapy, for the purpose of prescribing rabeprazole (RPZ) + AMPC + metronidazole (MNZ) for the RM. This study compared the overall *H. pylori* eradication rates between the periods with and without routine genetic testing of *CYP2C19*.

SUBJECTS AND METHODS

Subjects

Subjects were 551 patients (228 males and 323 females) aged 20 to 80 years who visited Daiko Medical Center between June 2004 and August 2010. *H. pylori* infection was examined using a ^{13}C-urea breath test and/or serum anti-*H. pylori* antibody. *CYP2C19* genotyping has been recommended to patients since November 2005. They were questioned about smoking habits at their first visit and recorded in their medical chart. For smokers, cessation was advised, at least during the period under medication, so as not to jeopardize the eradication. The follow-up study was approved by the Ethics Committee of the Nagoya University School of Medicine (approval number 155).

H. pylori eradication therapy

During the period without routine genetic testing of *CYP2C19*, a one-week regimen of LPZ 30 mg b.i.d + AMPC 750 mg b.i.d + CAM 200 mg b.i.d (LAC regimen) was administered for patients seeking eradication for the first time. For those failing at the other clinics, a different one-week regimen of LPZ 30 mg b.i.d + AMPC 750 mg b.i.d + MNZ 250 mg b.i.d (LAM regimen) was administered.

During the routine genetic testing period for *CYP2C19*, a one-week RAM regimen (RPZ 10 mg b.i.d + AMPC 750 mg b.i.d + MNZ 250 mg b.i.d) or LAM regimen was administered to RM. For those with the other genotypes, the LAC regimen was administered, except for patients with a history of drug allergy or of adverse effects from any LAC drug. When the LAC regimen was unsuccessful, the RAM regimen was prescribed for failures. The failures at other clinics with the LAC regimen were treated with the RAM regimen, LAM regimen, RMM regimen (RPZ 10 mg b.i.d + MNZ 250 mg b.i.d + minomycin (MNM) 100 mg b.i.d) or RAMM regimen (RPZ 10 mg b.i.d + AMPC 750 mg b.i.d + MNZ 250 mg b.i.d + MNM 100 mg b.i.d).

Genotyping of CYP2C19

Two single nucleotide polymorphisms of *CYP2C19*, G681A and G636A, were genotyped by a polymerase chain reaction with confronting two-pair primers (PCR-CTPP).[9,10] The **1* allele was denoted as *681G* and *636G*, the **2* allele as *681A* and *636G*, and the **3* allele as *681G*

and *636A*. DNA was extracted from a buffy coat by a BioRobot® EZ1 (QIAGEN Group, Tokyo) for the genotyping of *CYP2C19* G681A and G636A. Genotyping was conducted separately for the two polymorphisms by PCR-CTPP. The primers for G681A were F1: 5' AGA GCT TGG CAT ATT GTA TCT, R1: 5' TAA GTA ATT TGT TAT GGG TTC CC, F2: 5' CCA CTA TCA TTG ATT ATT TCC CA, and R2: 5' TCG ATT CTT GGT GTT CTT TTA C, and those for G636A were F1: 5' AAC CAG CTA GGC TGT AAT TGT, R1: 5' CTT GGC CTT ACC TGG ATC, F2: 5' ATT GTA AGC ACC CCC TGA, and R2: 5' CAC TGA TCA GGG AGC TAA TG. Those underlined are the bases with the single nucleotide polymorphism. Genomic DNA (30 ng to100 ng) was used per 25 μl of reaction with 0.18 mM dNTPs, 12.5 pmol of each primer, 0.5 units of "AmpliTaq Gold", and 2.5 μl GeneAmp 10×PCR Buffer including 15 mM $MgCl_2$ (Perkin-Elmer Corp., Foster City, CA). Amplification conditions were 10 minutes of initial denaturation at 95°C, followed by 30 cycles of 1 minute at 95°C, 1 minute at 59°C for G681A and 58°C for G636A, and 1 minute at 72°C, followed by a final 5-minute extension at 72°C. The amplified DNA was visualized on a 2% agarose gel with ethidium bromide staining. The alleles were distinguished as follows: a 131-bp band for *681G* allele and a 105-bp band for *681A* allele, with a 191-bp common band, and a 377-bp band for *636G* allele and a 255-bp band for *636A* allele, with a 597-bp common band.

Statistical analysis

The Hardy-Weinberg equilibrium was examined with a chi-square test. The percentage differences between the two groups were examined by a chi-square test. Each odds ratio (OR) and its 95% confidence interval (CI) were calculated by an unconditional logistic model. Statistical analysis was performed using Stata/MP 11 software (StataCorp LP, Texas, USA).

RESULTS

Table 1 shows the characteristics of subjects. There were more female patients than males. Those aged 50 years or older were dominant. Current smokers were 10.8% during the period without genotype testing, and 7.0% during the period with genotype testing. The genotype frequencies of *CYP2C19* among the subjects untreated before a visit during the period of routine genetic testing were 34.2% for **1*1*, 36.0% for **1*2*, 12.6% for **1*3*, 9.3% for **2*2*, 6.5% for **2*3*, and 1.4% for **3*3*. The allele frequencies were 0.584 for **1*, 0.306 for **2*, and 0.110 for **3*. The genotype frequencies of *CYP2C19* G681A and G636A were in Hardy-Weinberg equilibrium (p=1.000 for G681A and p=1.000 for G636A).

Table 2 describes the eradication rates among those firstly treated at the Daiko Medical Center. The overall rate among those evaluated for eradication was 80.0% (n=90) for the period without routine genetic testing, and 88.7% (n=124) for the period with testing; the difference was not significant (χ^2=3.11, p=0.078). The eradication rate for the RM was 100% (n=49) during the testing period. Three infected patients who did not want the genotype information were treated with the LAC regimen. The age-sex adjusted OR of eradication success was 2.29 (95% CI, 0.99–5.28, p=0.051) for the latter period relative to the former period among those aged less than 70 years, excluding three untested for *CYP2C19* during the latter period. The second treatment was provided for 25 failures: one of 12 during the former period and one of 13 during the latter period failed again (Table 2).

Table 3 shows the eradication rate of therapy for failures at the other clinics during each period. Those failures during the period without routine genetic testing for *CYP2C19* numbered 11 patients, seven of whom were treated with LAC or LAM regimens, and four of whom declined

Table 1 Characteristics of subjects

Characteristics		2004–2005			2005–2010			
		Untreated before visit		Failures at other clinics	Untreated before visit		Failures at other clinics	
		Infected	Uninfected	Infected	Infected	Uninfected	Infected	Uninfected
Sex	Male	62	23	4	73	40	22	4
	Female	68	42	7	122	49	33	2
Age	20–29	4	11	1	1	14	0	0
	30–39	9	11	1	18	23	9	0
	40–49	22	12	1	24	20	11	0
	50–59	57	21	5	59	17	16	3
	60–69	32	9	2	82	12	14	3
	>70	6	1	1	11	3	5	0
CYP2C19	**1*1*	N.A[a)]	N.A	N.A	66	29	24	3
	**1*2*	N.A	N.A	N.A	69	31	21	1
	**1*3*	N.A	N.A	N.A	27	8	4	0
	**2*2*	N.A	N.A	N.A	14	12	1	0
	**2*3*	N.A	N.A	N.A	13	5	0	1
	**3*3*	N.A	N.A	N.A	3	1	0	0
	Not tested	-	-	-	3	3	5	1
Smoking	Never	95	55	8	155	71	45	2
	Former	20	4	1	32	9	3	2
	Current	15	6	2	7	9	7	1
	Unknown	0	0	0	1	0	0	1
Smoking[b)]	Never	-	-	-	50	22	22	2
	Former	-	-	-	13	3	1	1
	Current	-	-	-	2	4	1	0
	Unknown	-	-	-	1	0	0	0
Total		130	65	11	195	89	55	6

a) N.A: Not applicable
b) Smoking history of those with **1*1* genotype

to undergo the treatment after hearing a detailed explanation. Among seven patients, five were successfully treated, while the remaining two did not visit the Center after medication. During the period of routine genetic testing for *CYP2C19*, 61 patients visited the Center, having experienced failure at the other clinics. Among them were 55 patients who were actually failures. Fifteen out of 55 patients were treated with LAC, RAM, LAM, RAMM, or RAM regimens. Except for 23 patients not evaluated after treatment, 26 out of 27 patients (96.3%) were successfully treated.

During the period of routine genetic testing for *CYP2C19* genotype, the proportion of those with the **1*1* genotype was 34.4% among 192 infected patients except for 3 untested in their initial therapy at the Center, and 48.0% among 50 infected failures at other clinics except for 5 untested. Although the difference was not statistically significant (χ^2=3.15, p=0.076), the proportion of those with **1*1* genotype was higher among the failures at other clinics.

Table 2 Eradication rate over the first treatment during the periods without (June, 2004 to October, 2005) and with (November, 2005 to August, 2010) a routine genetic test for *CYP2C19*

Subjects	2004–2005	2005–2010			
	Untested	**1*1*	Other genotypes	Untested*	Total
	n (%)	n (%)	n (%)	n (%)	n (%)
Visited	195 (100)	-	-	-	284 (100)
Infected	130 (66.7)	66	126	3	195 (68.7)
Treated	114	60	118	3	181
Succeeded	72 (80.0**)	49 (100.0**)	60 (82.2**)	1(50.0)	110 (88.7**)
Failed	18 (20.0**)	0	13 (17.8**)	1(50.0)	14 (11.3**)
Not evaluated	24	11	45	1	57
Re-treated	12	-	12	1	13
Succeeded	7 (87.5**)	-	9 (90.0)	-	9 (90.0**)
Failed	1 (12.5**)	-	1 (10.0)	-	1 (10.0**)
Not evaluated	4	-	2	1	3

*Untested before prescription

** Rate excluding those unevaluated

Table 3 Eradication rate from therapy for the failures at other clinics during the periods without (June, 2004 to October, 2005) and with (November, 2005 to August, 2010) a routine genetic test for *CYP2C19*

Subjects	2004–2005	2005–2010			
	Untested	**1*1*	Other genotypes	Untested	Total
	n (%)	n (%)	n (%)	n (%)	n (%)
Visited	11 (100)	-	-	-	61 (100)
Infected	11 (100.0)	24	26	5	55 (90.2)
Treated	7	19	26	5	50
Succeeded	5 (100.0*)	12 (100.0*)	12 (92.3*)	2 (100.0*)	26 (96.3*)
Failed	0	0	1 (7.7*)	0	1 (3.7*)
Not evaluated	2	7	13	3	23

* Rate excluding those unevaluated

DISCUSSION

In the present study, changes in the *H. pylori* eradication rate were compared between the periods with and without routine genetic testing for *CYP2C19*. The overall eradication rate for those first treated at the Center was 80.0% (n=90) in the former period and 88.7% (n=124) in the latter period; the difference was marginally significant (χ^2=3.11, p=0.078). The eradication rate for the RM was 100% (n=49). The age-sex adjusted OR of eradication success was also marginally significant (p=0.051 by two-sided test) among those less than 70 years of age, excluding three untested for *CYP2C19* during the latter period.

So far, a few studies have reported a significant association between the *H. pylori* eradication

rate and *CYP2C19* genotype (reduced eradication rate for RM),[8,10] while no such association was observed in other studies.[5-7] A meta-analysis revealed that the *CYP2C19* genotype was the main contributor, at least among those treated with LPZ.[11] Accordingly, we introduced routine *CYP2C19* genotyping to identify the RM, based on the assumption that the RAM regimen is more effective for the RM than the LAC regimen. The present study found that the eradication rate of the RAM regimen was 100% among 60 RM subjects. It was not clear whether RPZ or MNZ contributed more to the improved eradication rate. However, since the LAC regimen which is less effective for RM is the first-line treatment at present in Japan, the selection of the RM by genotype testing seemed one of the more reasonable options. The finding that the proportion of those with **1*1* genotype was higher among the failures at other clinics than among those infected in the first therapy at the Center (χ^2=3.15, p=0.076) indicated that those with that genotype were resistant to eradication therapy, mainly via the LAC regimen.

CAM-resistance of *H. pylori* is another important factor in treatment failure; the eradication rate for CAM-resistant strains with PPI/AMPC/CAM regimens were reported to range from 30 to 50%.[12,13] The lowered eradication rate was especially marked for the RM,[14] indicating that the regimens without CAM were preferable for the RM. One study reported that the proportion of CAM-resistant strains in Tokyo had risen from 6.2% in 1995 to 22.1% in 2001.[15] The Japanese Society for *Helicobacter* Research reported an increase from 18.9% in 2002 to 27.7% in 2004.[16,17]

One of the limitations of this observational study was the small number of study subjects, which rendered the difference in the eradication rate between the two periods to be insignificant as a whole. In order to detect a significant difference between 80% and 90% with two-sided α=0.05 and power=80%, 219 infected patients per period were required. Another limitation was the lack of information on the CAM-resistance of *H. pylori*. Since the Daiko Medical Center is a clinic for preventive services, gastroscopy was not available with the result that the resistance tests could not be conducted.

In conclusion, this study documented an improvement in the overall eradication rate through the introduction of a routine genetic test for *CYP2C19*, although the effects were marginally significant only among those less than 70 years of age. Since the cost-effectiveness for the genotyping procedure is an important issue in practice, studies on economical improvements will be required.

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