

RESEARCH ARTICLE

Effect of IL-1 Polymorphisms, CYP2C19 Genotype and Antibiotic Resistance on *Helicobacter pylori* Eradication Comparing Between 10-day Sequential Therapy and 14-day Standard Triple Therapy with Four-Times-Daily-Dosing of Amoxicillin in Thailand: a Prospective Randomized Study

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Abstract

Background: Studies of effects of IL-1 polymorphisms, CYP2C19 genotype together with antibiotic resistance for *H. pylori* eradication are rare worldwide. The present study was designed to evaluate efficacy of 10-day sequential therapy (SQT) and 14-day standard triple therapy (STT) with four- times-daily dosing of amoxicillin for *H. pylori* eradication related to these important host and bacterial factors in Thailand. **Materials and Methods:** This prospective randomized study was performed during March 2015 to January 2016. *H. pylori* infected gastritis patients were randomized to receive 10-day sequential therapy and 14-day standard triple therapy. CYP2C19 genotyping, IL1 polymorphism (IL-1B and IL-1RN genotypes) and antibiotic susceptibility tests were performed in all patients. 13C-UBT was conducted to confirm *H. pylori* eradication at least 4 weeks after treatment. **Results:** A total of 100 patients (33 males and 67 females, mean age=51.1 years) were enrolled. Eradication rate by PP analysis was 97.9% (47/48) with the 10-day SQT regimen and 87.8% (43/49) with 14-day STT regimen (97.9% vs 87.8%; p-value=0.053). Antibiotic susceptibility testing demonstrated 45% resistance to metronidazole, 14.8% to clarithromycin, and 24.1% to levofloxacin. CYP2C19 genotyping revealed 44.9% RM, 49% IM and 6.1% PM. IL-1B and IL-1RN genotypes were demonstrated as 21.4% for CC, 48.1% for TC, 36.8% for TT, 72.7% for 1/1, and 21.2% for 1/2 genotypes, respectively. The 10-day SQT regimen provided 100% eradication in patients with clarithromycin or dual clarithromycin and levofloxacin *H. pylori* resistant strains. Moreover, the 10-day SQT regimen resulted in a 100% eradication rate in all patients with CYP2C19 genotype RM and almost type of IL-1B (TC and TT) and IL1-RN genotypes (1/2 and other). **Conclusions:** Treatment with 10-day sequential therapy is highly effective for *H. pylori* eradication regardless of the effects of clarithromycin resistance, dual clarithromycin and levofloxacin resistance, CYP2C19 genotype, IL-1B and IL1-RN genetic polymorphisms and can be used as effective first line therapy in Thailand.

Keywords: IL-1 polymorphisms - dosing scheme of amoxicillin triple therapy -10-day sequential therapy

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Introduction

Helicobacter pylori (*H. pylori*) infection is the main cause of peptic ulcer disease (PUD), mucosa associated lymphoid tissue lymphoma, and gastric cancer (Vilaichone and Mahachai, 2001; Vilaichone et al., 2006). Furthermore, *H. pylori* eradication potentially reduces the incidence of gastric cancer, especially in high prevalence area of this particular cancer (Ford et al., 2014; Demirel et al., 2013). However, the success of eradication regimens depends on

many factors including compliance, antibiotic resistance, and proper dosing of proton pump inhibitor (PPI) that provides adequate acid suppression (Mahachai et al., 2011)

The global eradication rate of standard triple regimen for *H. pylori* infection has declined in recent years and no longer recommended as an empiric choice in most countries (Chey and Wong, 2007; Mahachai et al., 2011). However, an extended duration of PPI and clarithromycin-containing triple therapy from 7 to 10-14 days improved the eradication rate by about 5-10% (Vilaichone et al,

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2006, Malfertheiner et al., 2012). Interestingly, recent study has demonstrated the positive effect of the dosing scheme of amoxicillin significantly influenced eradication rates of triple therapies. Eradication rates in regimens with tid and qid dosing of amoxicillin were higher than those of regimens with the bid dosing of amoxicillin (Furuta et al., 2014). One of the popular present first-line treatment options is sequential regimen, which has shown higher eradication rates than conventional triple therapy in several recent meta-analyses (Zullo et al., 2007; Jafri et al., 2008; Gatta et al., 2009). IL-1 polymorphisms and CYP2C19 genotype together with antibiotic resistance of *H. pylori* markedly affect the eradication rate but rarely reports worldwide.

We conduct this study for evaluated efficacy of 10-day sequential therapy (SQT) and 14-day standard triple therapy (STT) with four times-daily dosing of amoxicillin for *H. pylori* eradication in Thai patients with functional dyspepsia as a first line *H. pylori* eradication in Thailand. We also evaluated antibiotic resistance pattern as well as the effect of IL-1 polymorphisms and CYP2C19 genotype with these two regimens.

Materials and Methods

Patients

Eligible patients age between 18-70 years who underwent gastroscopic examination at Thammasat University Hospital, Pathumthani, Thailand for dyspeptic symptoms during March 2015 and January 2016 were enrolled. After the endoscopy, those with diagnosis of non-ulcer dyspepsia, which was established during gastroscopy with normal finding or mild gastritis, were considered entering in this study. Exclusion criteria included patients with (1) a history of previous *H. pylori* eradication, (2) previous gastric surgery (3) currently on anticoagulants drugs, (4) administration of antibiotics, bismuth and PPI drugs in the preceding 4 weeks, (5) pregnancy, breast-feeding woman, (6) allergy to any one of the given medication in the regimens, and (7) other serious comorbidities (organ failure, malignancies). All patients provided written informed consent.

The diagnosis of *H. pylori* infection

During the endoscopy, 4 biopsy samples from gastric antrum were obtained for rapid urease test, *H. pylori* culture and Epsilometer test (E-test) or GenoType®HelicoDR, histological examination, IL-1 polymorphisms and CYP2C19 genotype. The results of CYP2C19 genotype testing were expressed as: rapid metabolizer (RM), intermediate metabolizer (IM) or poor metabolizer (PM). The results of IL1 polymorphisms (IL-1B and IL-1RN genotypes) testing was expressed as: CC, TC, TT, 1/1, 1/2, and other genotypes, respectively. The presence of *H. pylori* was defined as: (1) positive *H. pylori* culture, or (2) positive tests (rapid urease test or histology).

Therapeutic regimens

Randomization was made by reference to a computer-generated list. The two different groups were given (1) 10-day SQT (rabeprazole 20 mg, amoxicillin 1,000 mg

twice daily for 5 days, followed by rabeprazole 20 mg twice daily, 1g long acting clarithromycin MR once daily, and metronidazole 400 mg three times-daily for 5 days) (2) 14-day STT with four times-daily dosing of amoxicillin (rabeprazole 20 mg twice daily, amoxicillin 500 mg four times-daily, and 1g long acting clarithromycin MR once daily for 14 days)

Post-therapy follow-up

After completion of therapy for at least 4 weeks, 13C-urea breath test (UBT) was performed for all individuals to determine *H. pylori* eradication. Successful eradication was defined as negative result from 13C-UBT. Pill count was observed, and drug consumption over 90% was defined as good adherence. Side effects were assessed by personal interview using questionnaires for each patient. The potential adverse reactions listed in the questionnaires were bitter taste, nausea and vomiting, palpitation, diarrhea, and skin rashes. New symptoms and exacerbation of pre-existing symptoms during the period of treatment were determined to be therapy-related adverse reactions. Serious adverse reactions were defined as symptoms that disturbed on patients' daily life.

Statistical analysis

We expected the eradication rate of 14-day STT with four times-daily dosing of amoxicillin as an empiric therapy to be $\geq 90\%$. Treatment success was defined as a cure rate of $\geq 95\%$ (i.e. grade A) as described in prior study (Graham et al., 2009), and failure as a cure rate of $< 90\%$ per protocol analyses. The demographic findings and frequencies of adverse events were compared using chi-squared, Fisher's exact and student's t-test. P-value < 0.05 was considered to be statistically significant. The study was conducted according to good clinical practice guideline, as well as the Declaration of Helsinki, and was also approved by our local ethics committee.

Results

Total of 100 patients were included in this study, 33 men and 67 women with a mean age of 51.1 years. All 100 patients were randomized in to 2 groups as previously described. The patients with 10-day SQT regimens were younger age than those of 14-day STT group. Others baseline demographic data are shown in Table 1. One

Table 1. Baseline Demographic Data of All Patients

Characteristics	14-day STT (50)	10-day SQT (50)	P-value
Age (yr) [range]	53.6 [29-70]	48.5 [22-69]	
Sex(N) male	17 (34%)	16(32%)	0.83
female	33 (66%)	34(68%)	
Comorbidity dis.			
T2DM	5(10%)	3 (6%)	0.46
HTN	14(28%)	8(16%)	0.15
DLP	9(18%)	8(16%)	0.79
H/O smoking	1 (2%)	2 (4%)	0.56
H/O alcohol consumption	2 (4%)	3 (6%)	0.64

patient in 14-day STT group was loss to follow up after complete treatment due to poor adherence. Two patients in 10-day SQT regimen were loss to follow up due to poor adherence and concerning about side effects of treatment regimen.

Eradication rate of treatment regimens

The eradication rates were calculated by both intention-to-treat (ITT) and per-protocol (PP) analyses as shown in Figure 1. The eradication rates by ITT analysis was 86% (43/50) with 14-day STT regimen and 94% (47/50) with 10-day SQT regimen. PP eradication results were 87.8% (43/49) and 97.9% (47/48) with 14-day STT and 10-day SQT regimen, respectively. The cure rate was higher in 10-day SQT regimen than those in 14-day STT regimen from both ITT and PP. Only PP eradication of 10-day SQT group could achieve cure rate of grade A ($\geq 95\%$) as detail in Figure 1.

Antibiotic susceptibility and *H. pylori* eradication rate

Antibiotic susceptibility tests were performed in 54 strains (40 from E-test and 14 from GenoType®HelicoDR), which demonstrated 45% of metronidazole resistant, 24.1% of levofloxacin resistant and 14.8% of clarithromycin resistant strains as detail in Figure 2. The 10-day SQT regimen provided 100% eradication in patients with clarithromycin or dual clarithromycin & levofloxacin *H. pylori* resistant strains as detail in Table 2.

Host factors and *H. pylori* eradication rate

CYP2C19 genotype tests were performed in 98 cases (48 from 14-day STT regimen and 50 from 10-day SQT regimen). The CYP2C19 genotype tests revealed 44.9% RM, 49% IM and 6.1% PM. The prevalence of CYP2C19

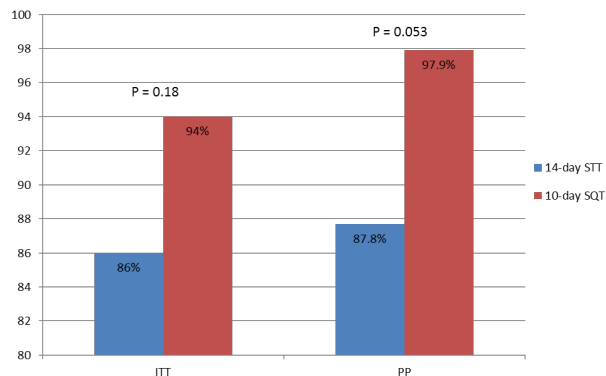


Figure 1. Eradication Rates with Both Regimens

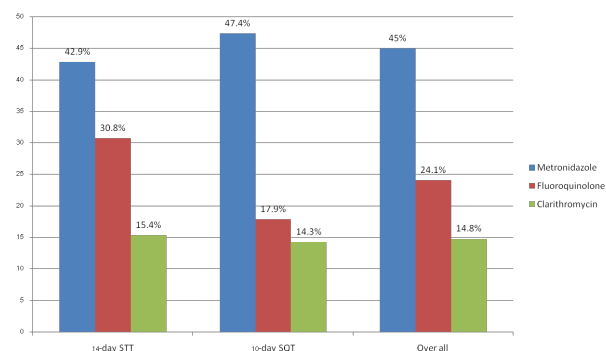


Figure 2. Prevalence of Antibiotic Resistance

genotype was similar in both groups as shown in Table 3.

IL-1B genotype were performed in 98 cases (48 from 14-day STT regimen and 50 from 10-day SQT regimen) and revealed 21.4% CC, 41.8% TC and 36.8% TT. IL-1RN genotype was also performed in 99 cases (49 from 14-day STT regimen and 50 from 10-day SQT regimens) and demonstrated 72.7% for genotype 1/1, 21.2% for genotype 1/2 and 6.1% for other genotypes. The result of IL-1 polymorphism and eradication rate were similar in all groups of patients. The 10-day SQT regimen has 100% eradication rate in all patients with CYP2C19 genotype RM, IM and almost type of IL-1B and IL-1RN genotypes as detailed in Tables 4 and 5.

Adverse events

The common side effects include bitter taste, nausea/vomiting, abdominal pain, diarrhea, headache, and fatigue which were found in all groups. Documented adverse reactions are shown in Table 6. None of subject experienced any major adverse event.

Table 2. Antibiotic Susceptibility and Eradication Rates for Each Regimen

Antibiotic susceptibility test	14-day STT	10-day SQT	P-value
Clarithromycin (C) resistance	2/4(50%)	3/3(100%)	0.15
Metronidazole (M) resistance	8/9(88.9%)	9/9(100%)	0.3
Fluoroquinolone (F) resistance	6/8(75%)	5/5(100%)	0.27
C + M resistance	1/2(50%)	0/0(0%)	0.12
C + F resistance	0/0(0%)	2/2(100%)	0.12
M + F resistance	2/2(100%)	1/1(100%)	0.12
C + M + F resistance	0/0(0%)	0/0(0%)	

Table 3. CYP2C19 Genotypes and Eradication Rates

CYP2C19 genotype (N=98)	14-day STT (48)	10-day SQT (50)	P-value
RM (N=44; 44.9%)	25 (92%)	19 (100%)	0.16
IM (N=48; 49%)	21 (81%)	27 (100%)	0.31
PM (N= 6; 6.1%)	2 (100%)	4 (75%)	0.43

Table 4. IL-1B Genotypes and Eradication Rates

IL1B (N=98)	14-day STT (48)	10-day SQT (50)	P-value
CC (N=21; 21.4%)	7 (85.7%)	14 (93%)	0.11
TC (N=41; 41.8%)	20 (80%)	21(100%)	0.97
TT (N=36; 36.8%)	21 (95 %)	15 (100%)	0.16

Table 5. IL-1 RN Genotypes and Eradication Rates

IL1RN (N=99)	14-day STT (49)	10-day SQT (50)	P-value
1/1 (N=72;72.7%)	38 (83.8%)	34 (96.9%)	0.29
1/2 (N=21;21.2%)	9 (100%)	12 (100%)	0.49
Other (N=6; 6.1%)	2 (100%)	4 (100%)	0.41

Table 6. Adverse Events with Each Regimen

Adverse events (N=97)	14-day STT (49)	10-day SQT (48)	P-value
Bitter taste	18(36.7%)	15(31.3%)	0.57
Nausea/vomiting	9(18.4%)	13(27.1%)	0.31
Abdominal pain	8(16.3%)	12 (25 %)	0.29
Diarrhea	6(12.2%)	8(16.7%)	0.54
Headache	5(10.2%)	4 (8.3%)	0.75
Fatigue	4 (8.2%)	8(16.7%)	0.2

Discussion

Currently, the eradication rate of *H. pylori* by STT was reported to be less than 70% in many countries worldwide, including Thailand (Vilaichone et al., 2006; Graham, 2009). Recent studies from Japan have demonstrated that the eradication rates in clarithromycin-based triple regimens with qid dosing of amoxicillin were higher than those of regimens with the bid dosing of amoxicillin about 14% (91.9% and 77.8% respectively.) (Furuta et al., 2014). The data on the effectiveness of the dosing scheme of amoxicillin triple therapy with clarithromycin-containing regimen in Thailand are limited. However, we reported the effectiveness of 14-day STT with four times-daily dosing of amoxicillin were only 86% by ITT and 87.8% by PP analyses which failed to achieve grade A eradication rate (>95%) and insufficient to be the first line therapy regimen in Thailand.

SQT has been tested mainly in Europe and found to have higher eradication rate than STT (Zullo et al., 2007). Recent study from Thailand has shown that a 10-day SQT is highly effective and well tolerated with 95% eradication rate. However, the eradication rate was drop to 57.1% with clarithromycin-resistant strains (Sirimontaporn et al., 2010). Our study has demonstrated that the efficacy of 10-day SQT were as high as 94% by ITT and 97.9% by PP analyses and could overcome the effect clarithromycin-resistant and dual clarithromycin-fluoroquinolone resistant strains. Metronidazole resistance has been reported approximately 35-50% in Thailand (Vilaichone et al, 2013; Srinarong et al, 2014; Prapitpaiboon et al, 2015; Vilaichone et al, 2015). Interestingly, 10-day SQT has also shown to overcome this common antibiotic resistant strain of *H. pylori* infection. Larger clinical trials need to confirm these important findings.

CYP2C19 genotype has recently been found to have an impact on *H. pylori* eradication, peptic ulcer healing, and therapeutic efficacy of PPI (Furuta et al., 2001; Yamada et al., 2001). This study demonstrated that genotype IM was the most common genotype same as prior studies in Thailand (Jainan et al, 2014; Srinarong et al, 2014). IL-1 polymorphisms (IL-1B and IL-1RN) have been consistent to increase gastric mucosal inflammation related to *H. pylori* infection by influenced gastric mucosal cytokine levels (Vilaichone et al., 2005). Rarely prior studies reported effect of IL-1 polymorphisms and CYP2C19 genotype and *H. pylori* eradication rate worldwide. Interestingly, the 10-day SQT regimen has high eradication rate (100%) in genotypes RM and IM of CYP2C19 and most genotype of IL-1 polymorphisms.

In summary, our study demonstrated high eradication rate (grade A) of *H. pylori* infection from 10-day SQT regardless of CYP2C19 genotype, clarithromycin or dual clarithromycin and fluoroquinolone resistant strains. Only minor side effects were observed from this regimen. Our study supports the idea that 10-day SQT can be used as effective first line therapy for *H. pylori* eradication in Thailand.

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