DR PING-I   HSU (Orcid ID : 0000-0003-3905-4674)

DR SENG-KEE   CHUAH (Orcid ID : 0000-0002-8934-3223)

DR DENG-CHYANG   WU (Orcid ID : 0000-0003-3742-0634)

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TITLE: Tetracycline-levofloxacin *versus* Amoxicillin-levofloxacin Quadruple Therapies in the Second-line Treatment of *H Pylori* Infection

AUTHORS:

Ping-I Hsu,[1] Feng-Woei Tsay,[2] John Y. Kao,[3] Nan-Jing Peng,[4], Yan-Hua Chen,[2] Sheng-Yeh Tang,[1] Chao-Hung Kuo,[5] Sung-Shuo Kao,[2] Huay-Min Wang,[2] I-Ting Wu,[1] Chang-Bih Shie,[1] Seng-Kee Chuah,[6] Deng-Chyang Wu,[5] On behalf of the Taiwan Acid-related Disease & Microbiota (TARD-M) Consortium

AUTHORS' AFFILIATIONS:

[1]Division of Gastroenterology and Hepatology, Department of Internal Medicine, An Nan Hospital, China Medical University, Tainan, Taiwan; [2]Kaohsiung Veterans General Hospital and National Yang-Ming University, Kaohsiung, Taiwan; [3]Division of Gastroenterology, Department of Internal Medicine, Michigan Medicine, University of Michigan, Ann Arbor, Michigan, USA; [4]Department of Nuclear Medicine, Taipei Veterans General Hospital and National Yang-Ming University, Taipei, Taiwan; [5]Division of Gastroenterology, Department of Internal Medicine, Kaohsiung Medical University Hospital, Kaohsiung Medical University, Kaohsiung, Taiwan, Taiwan; [6]Department of Internal Medicine, Kaohsiung Chang Gung Memorial Hospital, and Chang Gung University College of Medicine, Kaohsiung, Taiwan Division of Gastroenterology, Department of Internal Medicine, Kaohsiung Municipal Ta-Tung Hospital, Kaohsiung, Taiwan

REPRINT REQUEST TO THE CORRESPONDING AUTHOR:

Dr. Deng-Chyang Wu, No. 100, Tz-You 1st Road, Kaohsiung City 807, Taiwan. E-mail: dechwu@yahoo.com; Dr. Sheng-Yeh Tang, Division of Gastroenterology and Hepatology, Department of Internal Medicine, An Nan Hospital, China Medical University, No. 66, Sec. 2, Changhe Rd., Annan Dist., Tainan City 70965, Taiwan; Tel: +886-6-3553111 Ext 3668. E-mail: cbshie@gmail.com.

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ABBREVIATIONS USED IN THIS PAPER: AL: amoxicillin-levofoxacin; CI: confidence interval; hetEM: heterogeneous extensive metabolizer; homEM: homogeneous extensive metabolizer; ITT: intention to treat; *H pylori*: *Helicobacter pylori*; NSAIDs: non-steroidal anti-inflammatory drugs; PM: poor metabolizer; PP: per protocol; PPI: proton pump inhibitor; TL: tetracycline-levofloaxacin.

ABSTRACT

Background: The Maastricht V/Florence Consensus Report recommends amoxicillin-fluoroquinolone triple or quadruple therapy as a second-line treatment for *H pylori* infection. An important caveat of amoxicillin-fluoroquinolone rescue therapy is poor

eradication efficacy in the presence of fluoroquinolone resistance. The study aimed to investigate the efficacies of tetracycline-levofloxacin (TL) quadruple therapy and amoxicillin-levofloxacin (AL) quadruple therapy in the second-line treatment of *H pylori* infection.

Methods: Consecutive *H pylori*-infected subjects after the failure of first-line therapies were randomly allocated to receive either TL quadruple therapy (tetracycline 500 mg QID, levofloxacin 500 mg QD, esomeprazole 40 mg BID, and tripotassium dicitrato bismuthate 300 mg QID) or AL quadruple therapy (amoxicillin 500 mg QID, levofloxacin 500 mg QD, esomeprazole 40 mg BID, and tripotassium dicitrato bismuthate 300 mg QID) for 10 days. Post-treatment *H pylori* status was assessed 6 weeks after the end of therapy.

Results: The study was early terminated after an interim analysis. In the TL quadruple group, 50 out of 56 patients (89.3%) had successful eradication of *H pylori* infection. Cure of *H pylori* infection was achieved only in 39 of 52 patients (69.6%) receiving AL quadruple therapy. Intention-to-treat analysis showed that TL quadruple therapy achieved a markedly higher eradication rate than AL quadruple therapy (95% confidence interval: 4.8% to 34.6%; *P* = 0.010). Further analysis revealed that TL quadruple therapy had a high eradication rate for both levofloxacin-susceptible and resistant strains (100% and 88.9%). In contrast, AL quadruple therapy yielded a high eradication for levofloxacin-susceptible strains (90.9%) but a poor eradication efficacy for levofloxacin-resistant strains (50.0%). The two therapies exhibited comparable frequencies of adverse events (37.5% *vs* 21.4%) and drug adherence (98.2% *vs* 94.6%).

Conclusions: Ten-day TL quadruple therapy is more effective than AL quadruple therapy in the second-line treatment of *H pylori* infection in a population with high levofloxacin resistance.

KEYWORDS: *Helicobacter pylori*; second-line treatment; tetracycline-levofloxacin quadruple therapy; antibiotic resistance**INTRODUCTION**

  Helicobacter pylori (*H pylori*) infect more than 50% of humans globally. It is the major cause of chronic gastritis, gastric ulcer, duodenal ulcer, gastric adenocarcinoma and gastric mucosa-associated lymphoid tissue lymphoma.[1,2] Eradicating *H pylori* can prevent the recurrence of peptic ulcer disease and decrease the incidence of gastric cancer.[3,4] A recent survey for *H pylori* eradication therapy revealed that 7-day standard triple therapy remained the most popular regimen in the Asia-Pacific region.[5] Most physicians in Taiwan, Japan, and Korea adopted 7-day and 14-day standard triple therapies in the first-line treatment of *H pylori* infection (62% and 21%, 100% and 0%, and 81% and 7%,

respectively). However, the prevalence of antibiotic resistance markedly increases with time worldwide, and the eradication rate of standard triple therapy has decreased to less than 80% in most countries worldwide.[6,7] Although several emerging first-line therapies including bismuth quadruple therapy and non-bismuth quadruple (sequential, concomitant or hybrid therapy) can improve the eradication rate of *H pylori* infection, eradication failure still occurs in 3-24% of infected patients.[8-10]

The Maastricht V/Florence Consensus Report,[12] American College of Gastroenterology (ACG) Clinical Guideline,[13] and Toronto Consensus for the Treatment of *Helicobacter pylori* Infection[14] recommend 14-day bismuth quadruple therapy or 14-day amoxicillin-fluoroquinolone triple/quadruple therapy as second-line therapy for *H pylori* infection. Meta-analyses demonstrated that amoxicillin-levofloxacin triple therapy and bismuth quadruple therapy had comparable eradication rates.[15] However, the former had fewer adverse effects than the latter.

An important caveat of levofloxacin-amoxicillin triple therapy is poor eradication efficacy in the presence of fluoroquinolone resistance.[13] In Taiwan, a progressively higher primary resistance rate was observed for clarithromycin (11.8% to 20.4%), metronidazole (25.6% to 42.3%), and levofloxacin (17.3% to 38.8%) from 2013 to 2019.[16] In the second-line treatment of *H pylori* infection, the rates of resistance to tetracycline, levofloxacin, and amoxicillin in Taiwan were 0-4%, 21-53%, and 0-8%, respectively.[17-20] The data indicate that both tetracycline and amoxicillin are good candidates for antibiotics adopted in rescue treatment of *H pylori* infection. Recently, a novel 10-day tetracycline-levofloxacin (TL) quadruple therapy has developed for rescue treatment of *H pylori* infection.[21] It consists of tetracycline, levofloxacin, bismuth and a proton pump inhibitor for 10 days. The pilot study demonstrated that TL quadruple therapy could achieve a very high eradication rate (95.8%) for salvage treatment of *H pylori* infection.[21] A subsequent randomized control study showed that 10-day TL quadruple therapy achieved a markedly higher eradication rate than 10-day PPI-amoxicillin-levofloxacin triple therapy in the rescue treatment of *H pylori* infection (98.0% *vs* 69.2%).[22]

Currently, it remains unclear that the higher eradication rate of 10-day TL quadruple therapy compared with PPI-amoxicillin-levofloxacin triple therapy is due to the additional use of bismuth or switching amoxicillin to tetracycline in the rescue regimen. We, therefore, designed the prospective, multicenter, randomized controlled trial to compare the efficacy of 10-day TL quadruple therapy and 10-day amoxicillin-levofloxacin (AL) quadruple therapy in the second-line treatment of *H pylori* infection.

PATIENTS & METHODS

Study population

The multicentre, open-label, randomized controlled trial was performed at the Kaohsiung Medical University Hospital, Kaohsiung Veterans General Hospital, and Kaohsiung Municipal Hsiao-Kang Hospital in Taiwan in accordance with the principles of good clinical practice from the Declaration of Helsinki. Consecutive *H pylori*-infected adult subjects (≥ 20 years old) with the failure of first-line anti-*H pylori* therapies were recruited for this study. The presence of *H pylori* after a first-line eradication treatment was defined as (1) a positive result of ^{13}C urea breath test, (2) positive results of both rapid urease test and histology, or (3) a positive result of culture. Criteria for exclusion included (a) patients with previous gastric surgery, (b) ingestion of antibiotics, PPI or bismuth within the prior 4 weeks, (c) the coexistence of serious concomitant illness (for example, decompensated liver cirrhosis, uremia), (d) pregnant or lactating women, and (e) patients with allergic history to the medications used.

All participants gave written informed consent before recruitment. This trial was approved by the Institutional Review Board in each hospital. It was registered as a standard randomized Clinical Trial (ClinicalTrials.gov.identifier: NCT03779087). All had access to the study data and reviewed and approved the final manuscript.

Study Protocol

Randomization

Using a permuted block randomization with a block size of four, we randomly allocated eligible subjects to receive one of the two regimens (1:1): 10-day TL quadruple therapy or 10-day AL quadruple therapy. An independent research assistant at the Kaohsiung Medical Hospital generated the computerized random number sequence. The sequence was concealed in an opaque envelope until the intervention was assigned. Envelopes were kept at the Kaohsiung Medical Hospital. After eligible patients gave their written informed consent, an independent study nurse in each center telephoned the research assistant to obtain patients' treatment allocation. All clinical investigators were blind to the randomization sequence.

Procedures

The eligible subjects were received either 10-day TL quadruple therapy (tetracycline [tetracycline HCl; Taiwan Veterans Pharm, Chungli, Taiwan; 500 mg QID], levofloxacin

[Cravit; Sanofi-Aventis, Taoyoun, Taiwan; 500 mg QD], esomeprazole [Nexium; AstraZeneca, Sodertalje, Sweden; 40 mg BID], and tripotassium dicitrato bismuthate [KCB; Swiss Pharm, Tainan, Taiwan; 300 mg QID]) or 10-day AL quadruple therapy (amoxicillin [amoxicillin Trahydrate; Yung Shin Pharm, Taichung, Taiwan; 500 mg QID], levofloxacin 500 mg QD, esomeprazole 40 mg BID, and tripotassium dicitrato bismuthate 300 mg QID) therapy. All drugs were taken one hour before meals or night sleep.

Before recruitment, *H pylori* status was determined by a urea breath test or endoscopy with rapid urease test, histology, and culture according to our previous studies.[23] The results of rapid urease tests (PRONTO DRY, Medical Instruments Corp., Brignals, France) were interpreted as positive if the color of the gel turned pink or red 1 h after examination at room temperature. Two biopsy specimens were taken from the lesser curvature sites of the antrum and the corpus for histologic examination. Additionally, one biopsy specimen from the antrum and another one from the body were obtained for the culture of *H pylori* [24] The organisms were identified as *H pylori* by Gram staining, colony morphology, and positive catalase, oxidase, and urease reactions. The E-test (AB Biodisk, Solna, Sweden) was used to evaluate the resistance to antibiotics according to MIC (minimum inhibitory concentration) values of > 0.5 mg/L, > 4 mg/L, and > 1 mg/L for amoxicillin, tetracycline, and levofloxacin respectively.[22,25]

Subjects also completed a standardized questionnaire for demographic data and medical history, including age, sex, history of smoking, history of alcohol, coffee, and tea consumption, history of non-steroidal anti-inflammatory drug (NSAID) use, and concomitant illness. Additionally, blood sampling for genotyping of *CYP2C19* was performed using the polymerase chain reaction-based restriction fragment length polymorphism.[26] Genotypes of *CYP2C19* were categorized into three groups: homogeneous extensive metabolizer (homEM; *CYP2C19*1/*CYP2C19*1); heterogeneous extensive metabolizer (hetEM; *CYP2C19*1/*CYP2C19*2 and *CYP2C19*1/*CYP2C19*3); poor metabolizer (PM; *CYP2C19*2/*CYP2C19*2, *CYP2C19*2/*CYP2C19*3, and *CYP2C19*3/*CYP2C19*3).

Subjects were asked to record symptoms during the treatment period in a diary card. They returned to the clinics for assessing drug adherence and adverse events at the end of week 2. Adverse events were prospectively assessed according to a 4-point scale system: none; mild (discomfort annoying but not interfering with daily life); moderate (discomfort sufficient to interfere with daily life); and severe (discomfort resulting in discontinuation of eradication therapy).[27] *H pylori* eradication was determined by a ^{13}C-urea breath test using infrared spectrometer 6 weeks after completion of treatment. All patients were asked to stop treatment

with PPI for at least 2 weeks before urea breath tests. ^{13}C-urea breath test was conducted after an overnight fast using the Proto Pylori kit (Isodiagnostika, Canada). The 75-mg ^{13}C-urea was dissolved in water. Baseline and 30 min breath samples were assessed with an infrared spectrometer. Positive results of the ^{13}C-urea breath test were defined as a computer-generated $\delta^{13}CO_2$ value $\geq$ 4 units and negative results as < 2.5 units (20). The subjects with inconclusive results underwent another urea breath test at least 4 weeks later until the results became conclusive. The technicians performing urea breath tests were blinded to the eradication therapies received by patients.

End points

The primary end point was the cure of *H pylori* infection, which was determined by the result of ^{13}C-urea breath test.[22] The secondary end points were the frequency of overall adverse events and drug adherence. Drug adherence was assessed by counting unused medication at the end of week 2. Poor adherence was defined as taking less than 80% of pills were taken.[27]

Statistical analysis

The primary end point was the eradication rate of anti-*H pylori* treatment. According to previous studies,[22,28] the eradication rates of TL quadruple therapy and AL quadruple therapy were 98% and 83%, respectively. At least 76 patients were required in each treatment group to obtain a 15% absolute difference of eradication rate with a type I error of 0.05 and a type II error of 0.1 in 2-sided tests. The analysis was conducted by intention-to-treat (ITT) and per-protocol (PP). The ITT population included all randomized subject who receive at least one dose of eradication drugs. Patients whose infection status was unknown following treatment were considered treatment failures in the ITT analysis. The PP analysis excluded patients with unknown *H pylori* status following therapy and those with poor drug adherence. The outcomes were analyzed by the chi-square test with Yates' correction or Fisher's exact test for categorical data and the Student's t-test for continuous variables. Interim analysis was planned for the Independent Data Monitoring Committee to perform every 6 months. To avoid unnecessary trial hazard to the patients assigned to either treatment, the investigators planned to stop this randomized controlled trial if the difference of eradication rate was shown to be significant with *P*-value $\leqq$ 0.01 in the interim analysis.

RESULTS

Characteristics of the study groups

From July 2018 through June 2020, a total of 112 *H pylori*-infected subjects were randomly assigned to receive either TL quadruple e therapy (*n* = 56) or AL quadruple therapy (*n* = 56). They were all included in the ITT analysis. During the study period, four interim analyses for eradication efficacies of treatment arms were conducted by the Independent Data Monitoring Committee. Because interim efficacy analysis revealed that the AL quadruple group had a remarkably lower eradication rate than the TL quadruple group (*P* = 0.010), the Independent Data Monitoring Committee advised early termination of the study.

The demographic data of the recruited subjects are summarized in **Table 1**. The two study groups had comparable able age, gender, history of smoking, alcohol drinking, coffee, and tea consumption, history of NSAID use, type of gastrointestinal disease, first-line eradication therapies, and *CYP2C19* genotypes. **Figure 1** demonstrates the patient disposition. Among these eligible subjects recruited for the study, three with poor adherence and one lost to follow up were excluded from PP analysis of eradication efficacy.

Eradication of **H pylori**

Table 2 shows the eradication rates of TL and AL quadruple therapies. In the TL quadruple group, 50 out of 56 patients (89.3%) had successful eradication of *H pylori* infection. Cure of *H pylori* infection was achieved only in 39 of 52 patients (69.6%) receiving AL quadruple therapy. ITT analysis showed that TL quadruple therapy achieved a markedly higher eradication rate than AL quadruple therapy (Difference: 19.7%; 95% confidence interval: 4.8% to 34.6%; *P* = 0.010).   PP analysis revealed that *H pylori* infection was cured in 89.1% (49/55) of the patients receiving TL quadruple therapy and 69.8% (37/53) of those receiving AL quadruple therapy. TL quadruple therapy was superior to AL quadruple therapy (difference, 19.3%; 95% CI, 4.5% to 34.1%, *P* =0.013).

Figure 2 illustrates the differences in eradication rates between TL and AL quadruple therapies in patients with eradication failure of standard triple therapy, non-bismuth quadruple (sequential, concomitant or hybrid) therapy, or other therapies (for example, high-dose dual therapy, bismuth quadruple therapy) in the first-line treatment of *H pylori* infection. Among the patients with eradication failure of standard triple therapy (*n* = 29), TL and AL quadruple therapies had comparable eradication rate by ITT (86.7% *vs* 76.2%; *P* = 0.674) or PP analyses (85.7% *vs* 80.0%; *P* = 1.000). Among the patients receiving non-bismuth quadruple therapy

(n = 48), TL quadruple therapy was statistically superior to AL quadruple therapy according to both ITT (91.7% *vs* 65.2%; *P* = 0.036) and PP analyses (91.7% *vs* 63.6%; *P* = 0.032). Among the patients receiving other or unknown first-line therapies (bismuth quadruple therapy, n = 5; high-dose dual therapy, n = 5; PPI-bismuth-clarithromycin-amoxicillin therapy, n = 6; unknown first-line regimen, n = 17), TL and AL quadruple therapies achieved comparable eradication rates by ITT (88.2% *vs* 66.7%; *P* = 1.98) or PP analyses (88.2% *vs* 63.6%; *P* = 0.174).

Adverse events and compliances

All the patients were included in the ITT analysis for adverse events and adherence. In total, 37.5% (21/56) of the TL quadruple therapy recipients and 21.4% (12/56) of those treated with AL quadruple therapy reported at least one adverse event during eradication therapy. The two therapies exhibited similar frequencies of overall adverse events (*P* = 0.062). **Table 3** lists the profiles of adverse events of the two eradication treatments. The TL quadruple group had a higher frequency of nausea than the AL quadruple group (23.2% *vs* 5.4%; *P* = 0.013). No significant differences were observed between groups in the frequencies of other adverse events. One patient in the TL quadruple group stopped anti-*H pylori* medication due to joint pain (n=1). Three of the patients in the AL quadruple group discontinued treatment because of diarrhea (n = 2) and skin rash (n = 1). All patients but four (one in the TL quadruple group and three in the AL quadruple group) took more than 80% of the assigned tablets. The two treatment groups displayed similar compliance rates (98.2% *vs* 94.6%; *P* = 0.618).

Impacts of bacterial antibiotic resistances and host* CYP2C19 *genotypes on eradication therapy

Table 4 displays the impacts of antibiotic resistances on eradication therapy. Thirty-seven *H pylori* strains (TL quadruple group: n = 16; AL quadruple group: n = 21) were isolated from 41 patients receiving endoscopy with bacterial culture on enrollment. The resistant rates for levofloxacin, amoxicillin and tetracycline were 51.4%, 0% and 0%, respectively. In the TL quadruple group, the *H pylori* eradication rates for the levofloxacin-susceptible and resistant strains were 100% and 88.9%, respectively. There were no differences in eradication rates between levofloxacin-susceptible and resistant strains (*P* = 1.000). In the AL quadruple group, the eradication rates of levofloxacin-susceptible and resistant strains were 90.9% and 50.0%, respectively. There

was a borderline difference in eradication rates between levofloxacin-susceptible and resistant strains ($P = 0.063$).

In the TL quadruple group, the eradication rates of the patients with homEM, hetEM and PM genotypes were 90.0% (18/20), 85.7% (18/21) and 100.0% (5/5), respectively. No differences in eradication rates among those with different *CYP2C19* genotypes existed ($P = 0.645$). In the AL quadruple group, the eradication rates of the patients with homEM, hetEM and PM genotypes were 63.2% (12/19), 66.7% (14/21) and 83.3% (5/6), respectively. There were also no differences in eradication rates among patients with homEM, hetEM and PM genotypes ($P = 0.652$).

DISCUSSION

The Maastricht V/Florence Consensus Report recommends amoxicillin-fluoroquinolone triple or quadruple therapy as a second-line treatment for *H pylori* infection.[12] In the current study, we tested the hypothesis that switching amoxicillin to tetracycline in the quadruple regimen of salvage treatment can increase eradication efficacy. The data of this head-to-head, randomized, controlled trial clearly showed that 10-day TL quadruple therapy had a markedly higher eradication rate than of 10-day AL quadruple therapy, whether using ITT (89.3.0% *vs* 69.6%) or PP analysis (89.1% *vs* 69.8%). The results indicate that switching amoxicillin to tetracycline in the second-line levofloxacin-containing quadruple regimen can increase the eradication efficacy of second-line treatment. The novel finding in this study was consistent with a previous randomized controlled trial,[22] in which 10-day TL quadruple therapy achieved a higher eradication efficacy than 10-day AL triple therapy in the rescue treatment of *H pylori* infection (98.0% *vs* 69.2%). Currently, bismuth-based quadruple therapy with metronidazole and tetracycline is another important second-line treatment for *H pylori* infection recommended by most national and international guidelines.[12-14] Whether TL quadruple therapy can achieve a higher eradication rate than bismuth-based quadruple therapy containing metronidazole and tetracycline remains unclear and needs further investigation.

According to several studies,[22,29] the eradication rate of fluoroquinolone-containing triple therapy was suboptimal. A meta-analysis of the randomized controlled trials revealed that PPI-levofloxacin-amoxicillin triple therapy provided a cure rate of 76% in the salvage treatment of standard clarithromycin-containing triple therapy.[30] Another review article showed that AL triple therapy achieved an overall eradication rate of 78% in the rescue treatment of *H pylori* infection following the failure of a non-bismuth quadruple therapy.[31] An

important drawback of levofloxacin-amoxicillin triple therapy is poor eradication efficacy in the presence of levofloxacin-resistant strains.[13,30] Liao et al. showed that the eradication rates of 14-day lansoprazole-amoxicillin-levofloxacin triple therapy for levofloxacin-sensitive and resistant strains were 97% and 38% respectively.[32] Bismuth has been used to improve *H pylori* treatment for its bactericidal effect.[33] A randomized controlled trial[32] demonstrated that 14-day AL quadruple therapy achieved a higher eradication rate than 14-day AL triple therapy for levofloxacin-resistant strains (70.6% *vs* 37.5%, respectively). A recent study from Taiwan reported that the rate of resistance to levofloxacin in the second-line treatment of *H pylori* infection was 51.6% in 2019.[16] In the current study, the resistant rate for levofloxacin of *H pylori* strains following the failure of first-line therapies was 51.4%. Although AL quadruple therapy could yield a high eradication rate for levofloxacin-susceptible strains (90.9%), its eradication efficacy for levofloxacin-resistant strains was poor (50.0%). In contrast, 10-day TL quadruple therapy could achieve a high eradication rate for both levofloxacin-susceptible and resistant strains (100% and 88.9%).

There is a trend of increasing resistance rates to levofloxacin worldwide.[36] The rate of resistance to levofloxacin in the second-line treatment of *H pylori* infection is $\geqq$ 15% in the majority of WHO regions.[37] Ideally, all antimicrobial therapies are susceptibility-based. However, cost and convenience dictate that a proven reliable highly effective empiric regimen would generally be preferred initially. The current study shows that 10-day TL quadruple therapy is an effective rescue therapy and can achieve a high eradication rate for both levofloxacin-susceptible and resistant strains. The results suggest that the novel therapy has a great potential to be applied in the salvage treatment of *H pylori* infection in areas with high levofloxacin resistance.

In this study, both TL quadruple and AL quadruple therapies were well tolerated and had comparable drug adherence (98.2% *vs* 94.6%). Also, the two therapies exhibited similar frequencies of overall adverse events (37.5% *vs* 21.4%). Though the TL quadruple group had a higher frequency of nausea than the AL quadruple group (23.2% *vs* 5.4%), the severities of nausea in all the subjects receiving TL quadruple therapy were mild to moderate.

The strengths of this trial included a comparison with a multi-center, randomized-controlled method. Additionally, the efficacies of two rescue treatments for levofloxacin-susceptible and -resistant strains were investigated. One limitation of this study is that it was conducted in a single country. The results will need to be confirmed in other countries with different profiles of antibiotic resistance. Secondly, the number of *H pylori*

strains with antibiotic resistance data was small because the urea breath test was adopted to determine the *H pylori* status following first-line therapy in most of the eligible patients. Thirdly, post-marketing drug surveillance of fluoroquinolones has uncovered an array of disabling side effects, ranging from tendon problems to nerve damage to deadly tears of the aorta.[34,35] Currently, the FDA warns that fluoroquinolones should only be used to treat certain types of mild to moderate infections if the benefits clearly outweigh the risks. Fourthly, the duration of both second-line therapies are not optimized. All the Maastricht V/Florence Consensus Report, ACG Clinical Guideline, and Toronto Consensus make a strong recommendation for the duration of rescue therapies of 14 days instead of 10 days used in this study.[12-14] The inabilities of both therapies to produce a higher cure rate was likely in part due to the shorter than recommended duration of therapy.

In conclusion, 10-day TL quadruple therapy is significantly more effective than 10-day AL quadruple therapy as a second-line treatment of *H pylori* infection in a population with high levofloxacin resistance. Because efficacies of anti-*H pylori* therapies are influenced by several critical components of eradication regimens such as dosing, duration, relative potency of the PPI, prevalence of resistance, further studies are warranted to investigate the eradication outcomes of TL quadruple therapy with different treatment duration and current recommended rescue therapies in other areas with various antibiotic resistances.

REFERENCES

1. Suerbaum S, Michetti P. *Helicobacter pylori* infection. *N Engl J Med.* 2002;347: 1175–1186.

2. Malfertheiner P, Chan FK, McColl KE. Peptic ulcer disease. *Lancet.* 2009;374:1449–1461.

3. Sung JJY, Chung SCS, Ling TKW, et al. Antibacterial treatment of gastric ulcer

associated with *Helicobacter pylori*. *N Eng J Med.* 1995;332:139-42.

4. Ford AC , Forman D , Hunt RH et al. *Helicobacter pylori* eradication therapy to prevent gastric cancer in healthy asymptomatic infected individuals: systematic review and meta-analysis of randomized controlled trials. *BMJ.* 2014;348:g3174.

5. Chuah YY, Wu CC, Chuah SK, et al. Real-world practice and Expectation of Asia-Pacific physicians and patients in Helicobacter Pylori eradication (REAP-HP Survey). Helicobacter 2017;22: doi: 10.1111/hel.12380.

6. Gumurdulu Y, Serin E, Ozer B, et al. Low eradication rate of *Helicobacter pylor*i with triple 7-14 days and quadruple therapy in Turkey. *World J Gastroenterol.* 2004;10:668-671.

7. Bigard MA, Delchier JC, Riachi G, et al. One-week triple therapy using omeprazole, amoxycillin and clarithromycin for the eradication of *Helicobacter pylori* in patients with non-ulcer dyspepsia: influence of dosage of omeprazole and clarithromycin. *Aliment Pharmacol Ther.* 1998;12:383-388.

8. Graham DY, Fischbach L. *Helicobacter pylori* treatment in the era of increasing antibiotic resistance. *Gut.* 2010;59:1143-1153.

9. Saleem N , Howden CW. Update on the management of Helicobacter pylori infection. *Curr Treat Options Gastroenterol.* 2020;1-12.

10. Hsu PI, Wu DC, Wu JY, et al. Modified sequential *Helicobacter pylori* therapy: proton pump inhibitor and amoxicillin for 14 days with clarithromycin and metronidazole added as a quadruple (hybrid) therapy for the final 7 days. *Helicobacter.* 2011;16:139-145.

11. Wu DC, Hsu PI, Wu JY, et al. Sequential and concomitant therapies with 4 drugs are equally effective for eradication of *H pylori* Infection. *Clin Gastroenterol Hepatol.* 2010;8:36-41.

12. Malfertheiner P, Megraud F, O'Morain CA, et al. Management of Helicobacter pylori infection — The Maastricht V/ Florence Consensus Report. *Gut.* 2017;66:6-30.

13. Chey WD, Leontiadis GI, Howden CW, et al. ACG Clinical Guideline: Treatment of *Helicobacter pylori* infection. *Am J Gastroenterol* 2017;112:212-238.

14. Fallone CA, Chiba N, van Zanten SV, et al. The Toronto consensus for the treatment of Helicobacter pylori infection in adults. *Gastroenterology* 2016;151:61-69.

15. Di Caro S, Fini L, Daoud Y, et al. Levofloxacin/amoxicillin-based schemes vs quadruple therapy for Helicobacter pylori eradication in second-line. *World J Gastroenterol.* 2012;18:5669–5678.

16. Liang CM, Tai WC, Hsu PI, et al. Trend of changes in antibiotic resistance

in Helicobacter pylori from 2013 to 2019: a multicentre report from Taiwan. *Therap Adv Gastroenterol* 2020;13:1756284820976990.

17. Wu IT, Chuah SK, Lee CH, et al. Five-year sequential changes in secondary antibiotic resistance of *Helicobacter pylori* in Taiwan. *World J Gastroenterol.* 2015;21:10669-10674.

18. Wu DC, Hsu PI, Chen A, et al. Randomized comparison of two rescue therapies for *Helicobacter pylori* infection. *Eur J Clin Invest.* 2006; 36:803-809.

19. Liou JM, Bair MJ, Chen CC, et al. Levofloxacin sequential therapy vs levofloxacin triple therapy in the second-line treatment of Helicobacter pylori: a randomized trial. *Am J Gastroenterol.* 2016;111:381-387.

20. Lin TF, Hsu PI. Second-line rescue treatment of Helicobacter pylori infection: Where are we now? *World J Gastroenterol.* 2018;24:4548-4553.

21. Hsu PI, Chen WC, Tsay FW, et al. Ten-day quadruple therapy comprising proton-pump inhibitor, bismuth, tetracycline, levofloxacin achieves a high eradication rate for *Helicobacter pylori* infection after failure of sequential therapy. *Helicobacter.* 2014;19:74-9.

22. Hsu PI, Tsai FW, Kao SS, et al. Ten-day quadruple therapy comprising proton pump inhibitor, bismuth, tetracycline, and levofloxacin is more effective than standard evofloxacin triple therapy in the second-line treatment of Helicobacter pylori infection: a randomized controlled trial. *Am J Gastroenterol.* 2017;112:1374-1381.

23. Hsu PI, Wu DC, Wu JY, et al. Is there a benefit to extending the duration of *Helicobacter pylori* sequential therapy to 14 days? *Helicobacter.* 2011;16:146-152.

24. Hsu PI, Wu DC, Chen WC, et al. Comparison of 7-day triple, 10-day sequential and 7-day concomitant therapies for *Helicobacter pylori* infection - a randomized controlled trial. *Antimicrob Agents Chemother.* 2014;214:5936-5942

25. Hsu PI, Tsay FW, Kao JY, et al. Equivalent efficacies of reverse hybrid and concomitant therapies in first-line treatment of Helicobacter pylori infection. *J Gastroenterol Hepatol.* 2020;35:1731-1737.

26. Kita T, Sakaeda T, Aoyama N, et al. Optimal dose of omeprazole for CYP2C19 extensive metabolizers in anti-*Helicobacter pylori* therapy: pharmacokinetic considerations. *Biol Pharm Bull.* 2002;25:923-927.

27. Wu DC, Kuo CH, Tsay FW, et al. A pilot randomized controlled study of dexlansoprazole MR-based triple therapy for Helicobacter pylori infection. *Medicine (Baltimore).* 2016;95:e2698.

28. Gao X-Z, Qiao X-L, Song W-C, et al. Standard triple, bismuth pectin quadruple and sequential therapies for Helicobacter pylori eradication. *World J Gastroenterol.* 2010;16:4357–4362.

29. Kuo CH, Hu HM, Kuo FC, et al. Efficacy of levofloxacin-based rescue therapy for *Helicobacter pylori* infection after standard triple therapy: a randomized controlled trial. *J Antimicrob Chemother.* 2009;63:1017-1024.

30. Marin AC, McNicholl AG, Gisbert JP. A review of rescue regimens after clarithromycin-containing triple therapy failure for *Helicobacter pylori* eradication. *Expert Opin Pharmacother.* 2013;14:843–861.

31. Gisbert JP, Molina-Infante J, Marin AC, et al. Second-line rescue triple therapy with levofloxacin after failure of non-bismuth quadruple 'sequential' or 'concomitant' treatment to eradicate H. pylori infection. *Gastroenterology.* 2014;146:S394.

32. Liao J, Zheng Q, Liang X, et al. Effect of fluoroquinolone resistance on 14-day levofloxacin triple and triple plus bismuth quadruple therapy. *Helicobacter.* 2013;18:373–377.

33. Graham DY, Lee SY. How to effectively use bismuth quadruple therapy: the good, the bad, and the ugly. *Gastroenterol Clin N Am.* 2015;44:537-563.

34. Weel I, Chin B, Syn N, et al. The association between fluoroquinolones and aortic dissection and aortic aneurysms: a systematic review and meta-analysis. *Sci Rep* 2021;11:11073.

35. Gatti M, Bianchin M, Raschi E, et al. Assessing the association between fluoroquinolones and emerging adverse drug reactions raised by regulatory agencies: An umbrella review. *Eur J Intern Med* 2020;75:60-70.

36. Thung I, Aramin H, Vavinskaya V, et al. The global emergence of Helicobacter pylori antibiotic resistance. *Aliment Pharmacol Ther* 2016;43:514-33.

37. Savoldi A, Carrara E, Graham DY, et al. Prevalence of antibiotic resistance in Helicobacter pylori: a systematic review and meta-analysis in World Health Organization regions. *Gastroenterology* 2018;155: 1372–82.

Table 1   Demographic data of tetracycline-levofloxacin (TL) and amoxicillin-levofloxacin (AL) quadruple groups

	TL quadruple	AL quadruple	*P*-value

Characteristics	group (n = 56)	group (n = 56)	
Age (yr) (mean ± SD)	57.7±9.5	56.8 ±11.1	0.656
Gender (male / female)	22/34	16/40	0.231
Smoking	4 (7.31%)	5 (8.9%)	1.000
Alcohol consumption	2 (3.6%)	1 (1.8%)	1.000
Ingestion of coffee	17 (30.4%)	18 (32.1%)	0.926
Ingestion of tea	10 (17.9%)	13 (23.2%)	0.483
NSAID user	0 (0%)	1 (1.8%)	1.000
Underlying diseases	17 (30.4%)	22 (39.3%)	0.321
Peptic ulcer disease	17 (30.4%)	16 (28.6%)	0.836
Type of first-line therapy			0.390
Standard triple therapy	15 (26.8%)	21 (37.5%)	
Non-bismuth quadruple therapy	24 (42.9%)	23 (41.1%)	
Others	17 (30.4%)	21 (21.4%)	
CYP2C19 genotype			0.943
Homo EM	20 (43.5%)	19 (41.3%)	
Het EM	21 (45.7%)	21 (45.7%)	
PM	5 (10.9%)	6 (13.0%)	

Table 2 Major outcomes of 10-day TL quadruple therapy and 10-day AL quadruple therapy

	Eradication Rate		
Eradication rate	TL quadruple therapy (n = 56)	AL quadruple therapy (n = 56)	*P*-value
Intention-to-treat	89.3% (50/56) (81.2% ~ 97.4)*	69.6% (39/56) (57.8% ~ 81.6%)	0.010

Per-protocol	89.1% (49/55)	69.8% (37/53)	0.013
	(80.9% ~ 97.3%)	(57.4% ~ 82.1%)	

* 95% confidence interval

Table 3   Adverse events of TL quadruple therapy and AL quadruple therapy

Adverse Events	TL quadruple therapy (n = 56)	AL quadruple therapy (n = 56)	P-value
Abdominal pain	2 (1/1/0)*	2 (1/1/0)	1.000
Constipation	1 (1/0/0)	0 (0/0/0)	1.000
Diarrhea	5 (2/3/0)	6 (3/1/2)	1.000
Dizziness	4 (3/1/0)	1 (1/0/0)	0.364
Taste perversion	3 (1/2/0)	3 (2/1/0)	1.000
Headache	1 (1/0/0)	2 (2/0/0)	1.000
Anorexia	2 (2/0/0)	0 (0/0/0)	0.495
Nausea	13 (4/9/0)	3 (0/3/0)	0.013
Vomiting	4 (3/1/0)	0 (0/0/0)	0.118
Skin rash	0 (0/0/0)	1 (0/0/1)	1.000
Fatigue	3 (2/1/0)	2 (1/1/0)	1.000
Others	3 (1/1/1)	1 (1/0/0)	0.618

* Numbers of patients who suffered from mild, moderate and severe adverse events.

Table 4   Impacts of bacterial antibiotic resistances on eradication therapy

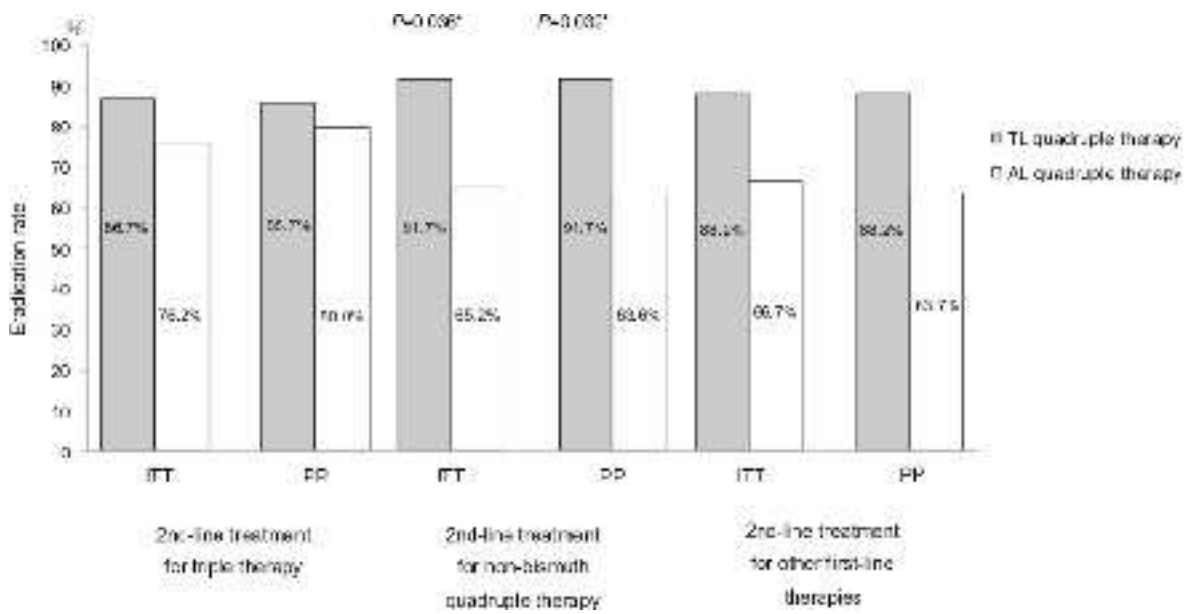| Antibiotic resistance	Eradication Rate		P-value
	TL quadruple therapy	AL quadruple Therapy	
Amoxicillin-resistant			
(-)	93.8% (15/16)	71.4% (15/21)	0.113
(+)	-	-	-
Tetracycline-resistant			
(-)	93.6% (15/16)	71.4% (15/21)	0.113
(+)	-	-	-
Levofloxacin-resistant			
(-)	100% (7/7)	90.9% (10/11)	1.000
(+)	88.9% (8/9)	50.0% (5/10)	0.141

Figure legend

Figure 1 Disposition of patients.

Figure 2 The differences in eradication rates between TL quadruple and AL quadruple therapies in patients with eradication failure of first-line standard triple therapy, non-bismuth quadruple therapy and other therapies.
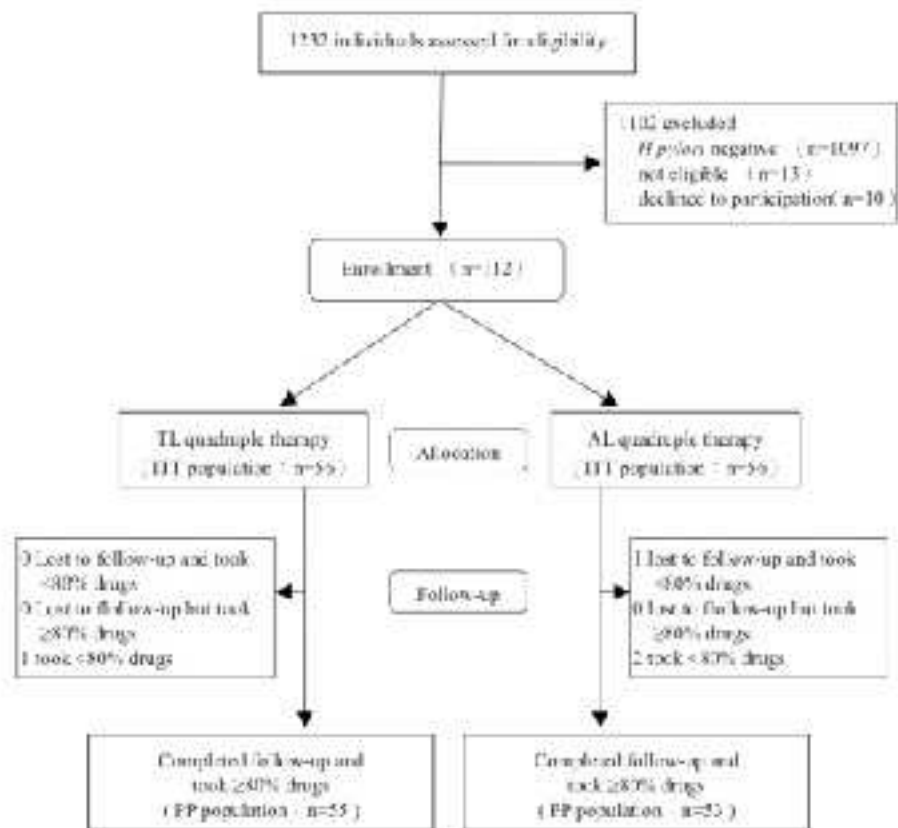
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