**Name of Journal: World Journal of Gastroenterology**

**ESPS Manuscript NO: 16501**

**Manuscript Type: ORIGINAL ARTICLE**

***Prospective Study***

**Hybrid *vs* sequential therapy for eradication of *Helicobacter pylori* in Taiwan: a prospective randomized trial**

Chen KY *et al*. Hybrid therapy for *H. pylori* eradication

Kuan-Yang Chen, Tsung-Jung Lin, Chin-Lin Lin, Hsi-Chang Lee, Chung-Kwe Wang, Deng-Chyang Wu

**Kuan-Yang Chen,** Institute of Clinical Medicine, National Yang-Ming University, Taipei City 10629, Taiwan

**Kuan-Yang Chen, Tsung-Jung Lin, Chin-Lin Lin, Hsi-Chang Lee, Chung-Kwe Wang,** Liver Center, Department of Gastroenterology, Ren-Ai Branch, Taipei City Hospital, Taipei City 10629, Taiwan

**Deng-Chyang Wu,** Division of Gastroenterology, Department of Internal Medicine, Kaohsiung Medical University Hospital, Kaohsiung City 80708, Taiwan

**Deng-Chyang Wu,** Division of Internal Medicine, Kaohsiung Municipal Hsiao-Kang Hospital, Kaohsiung Medical University, Kaohsiung City 80708, Taiwan

**Deng-Chyang Wu,** Center for Stem Cell Research, Kaohsiung Medical University Hospital, Kaohsiung City 80708, Taiwan

**Deng-Chyang Wu,** Department of Medicine, Faculty of Medicine, College of Medicine, Kaohsiung Medical University, Kaohsiung City 80708, Taiwan

**Deng-Chyang Wu,** Center for Infectious Disease and Cancer Research, Kaohsiung Medical University, Kaohsiung City 80708, Taiwan

**Author contributions:** Chen KY and Wu DC conceived and designed the study; Chen KY, Lin TJ, Lin CL, Lee HC and Wang CK performed the experiments; Chen KY and Lee HC analyzed the data; Chen KY and Lee HC wrote and revised the paper.

**Institutional review board statement:** All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008, and was reviewed and approved by the Institutional Review Board at Taipei City Hospital.

**Informed consent statement:** All participants were informed about the purpose and general procedures of this study and informed consents were signed prior to study enrollment.

**Conflict-of-interest statement:** The authors (Chen KY, Lin TJ, Lin CL, Lee HC, Wang CK and Wu DC) declare no conflict of interest relevant to this study.

**Data sharing statement:** No additional data are available.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: http://creativecommons.org/licenses/by-nc/4.0/

**Correspondence to: Hsi-Chang Lee, MD,** Liver Center, Department of Gastroenterology, Ren-Ai Branch, Taipei City Hospital, No. 10, Sec4 Ren-Ai Rd, Da-An Dist, Taipei City 10629, Taiwan. dab73@tpech.gov.tw

**Telephone:** +886-2-27093600

**Fax:** +886-2-27047859

**Received:** January 20, 2015

**Peer-review started:** January 21, 2015

**First decision:** February 10, 2015

**Revised:** April 12, 2015

**Accepted:** July 8, 2015

**Article in press:**

**Published online:**

**Abstract**

**Aim:** To investigate the efficacies of sequential and hybrid therapy in patients with *Helicobacter pylori* (*H. pylori*) infection.

**Methods**: From March 2013 to May 2014, one hundred and seventy-five *H. pylori* infected patients who had not been treated for *H. pylori* before were randomized to receive either sequential therapy (rabeprazole, 20mg and amoxicillin, 1 g twice daily for 5 d, following by rabeprazole 20 mg, clarithromycin 500 mg and metronidazole 500 mg twice daily for 5 d) or hybrid therapy (rabeprazole, 20 mg and amoxicillin 1 g for 7 d, following by rabeprazole 20 mg, amoxicillin 1 g, clarithromycin 500 mg and metronidazole 500 mg twice daily for 7 d). *H. pylori* status was confirmed by positive results of both rapid urease test and histology examination or a positive result of culture. Eradication efficacy was assessed by follow-up endoscopy with rapid urease test and histological examination 8 wk after the end of anti-*H. pylori* therapy, or 13C-urea breath test at least 4 wk after completion of treatment. The primary outcome was *H. pylori* eradication by intension-to-treat (ITT) and per-protocol (PP) analyses.

**Results**: One hundred and sixty-seven patients (83 patients in sequential group and 84 patients in hybrid group) completed the study. The compliance rates were 97.6% and 97.7% for the two groups, respectively. The eradication rate was 78.2% for the sequential group and 92% for the hybrid group by ITT analysis (*p =* 0.01). The eradication rate was 81.9% for the sequential group and 96.4% for the hybrid group by PP analysis (*p =* 0.01). Univariate analysis for the clinical and bacterial factors did not identify any risk factors associated with treatment failure. Severe adverse events were observed in 2.3% of patients in the sequential group and 2.4% of those in the hybrid group.

**Conclusion:** Due to a grade A (> 95%) success rate for *H. pylori* eradication by PP analysis, similar compliance and side events, hybrid therapy seems to be an appropriate eradication regimen in Taiwan.

**Key words:** *Helicobacter pylori*; Sequential therapy; Hybrid therapy

**© The Author(s) 2015.** Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** The ideal therapy regimen for *Helicobacter pylori* (*H. pylori*) infection should achieve an eradication rate ≥ 90% on per-protocol analysis. Both the hybrid and sequential therapy have shown eradication rates superior to the standard triple therapy in several studies. To the best of our knowledge, this is the first study on the comparison between hybrid therapy and sequential therapy in Taiwan population. Hybrid regimen is more effective therapy for *H. pylori* eradication than sequential regimen.

Chen KY, Lin TJ, Lin CL, Lee HC, Wang CK, Wu DC. Hybrid *vs* sequential therapy for eradication of *Helicobacter pylori* in Taiwan: a prospective randomized trial. *World J Gastroenterol* 2015; In press

**Introduction**

*Helicobacter pylori* (*H. pylori*) infection is very common worldwide, occurring in 40% to 50% of the population in developed countries, in 80% to 90% of the population in developing regions[[1](#_ENREF_1),[2](#_ENREF_2)], and about 50% of the population in Taiwan[[2](#_ENREF_2)]. The *H. pylori* infection causes chronic gastritis which significantly increases the risk of developing gastric or duodenal ulcers[[3](#_ENREF_3),[4](#_ENREF_4)], gastric adenocarcinoma, and mucosa-associated lymphoid tissue (MALT) lymphoma[[5](#_ENREF_5),[6](#_ENREF_6)]. Eradication of *H. pylori* infection prevents ulcer recurrence[[7](#_ENREF_7),[8](#_ENREF_8)], leads to a significant reduction of gastric cancer, decreases intestinal type gastric cancer recurrence in patients undergone endoscopic resection[[9](#_ENREF_9)], and results in complete regression of 60%-83% of MALToma[[10](#_ENREF_10)].

Until recently, the gold standard regimen for *H. pylori* eradication consisted of triple therapy with a proton pump inhibitor (PPI) plus clarithromycin and amoxicillin or metronidazole, administered for 7-14 d[[11](#_ENREF_11),[12](#_ENREF_12)]. The failure rates of these standard therapies range from 5% to 35%[[13-15](#_ENREF_13)]. The main reasons for eradication failure are poor patient compliance, resistant bacteria, low gastric pH and a high bacterial load[[16](#_ENREF_16),[17](#_ENREF_17)].

Sequential therapy, as originally defined, starts with a simple double regimen of a PPI plus amoxicillin for 5 d, followed by a triple regimen of a PPI, clarithromycin, and tinidazole for the next 5 d[[18](#_ENREF_18)]. Recent studies with antimicrobial susceptibility testing have confirmed that the superiority of sequential therapy over standard triple therapy is primarily because of an improved outcome with clarithromycin-resistant strains[[19](#_ENREF_19)]. However, sequential therapy was not demonstrated to achieve 95% of per-protocol (PP) eradication rate.

In 2011, Hsu *et al*[[20](#_ENREF_20)] report study using a hybrid regimen, starting with PPI plus amoxicillin for 7 d, and followed by a quadruple regimen of a PPI, amoxicillin, clarithromycin, and metronidazole for the next 7 d. The eradication rate was 99.1% by per-protocol (PP) analysis and 97.4% by intension-to-treat (ITT) analysis. The PP eradication rates achieve grade A success based on grading success (≥ 95% = A, 90%-94% = B, 86%-89% = C, and ≤ 85% = F[[21](#_ENREF_21)]).

Fewer studies compared sequential therapy with hybrid therapy. Two studies from Italy and Iran that compared sequential therapy for 10 d with hybrid therapy for 14 d showed contradictory results[22,23]. The reason was unknown because susceptibility tests were not done. Therefore, we did a randomized controlled trial to compare sequential regimen and hybrid regimen for the treatment of *H. pylori* infection. We accessed the antibiotic resistance that might affect the eradication rate. In Taiwan, the resistance rate of metronidazole is generally high and that of clarithromycin is increasing, allowing the opportunity to compare sequential therapy and hybrid therapy in patients with single and dual antibiotic resistance.

**MATERIALS AND METHODS**

***Setting and participants***

We surveyed patients who the gastroenterology clinics of Taipei city hospital between March 2013 and May 2014. Patients with *H. pylori* infection were enrolled in this study. Pre-enrollment procedures included biopsy of the gastric mucosa where the presence of *H. pylori* was assessed by rapid urease test, culture and histological examination of the tissue. The presence of *H. pylori* was defined as: (1) positive results of both rapid urease test and histology examination; or (2) a positive result of culture. Blood samples were taken for laboratory tests including renal and liver function tests to ascertain that there were no abnormal tests that wound preclude entry into the trial.

Criteria for exclusion included: (1) use of antibiotics within the preceding 30 d; (2) regular use of a PPI (> 3 times per week) in the 30 d before enrollment; (3) previous surgery of the stomach; (4) patients previously treated for *H. pylori* infection; (5) use of concomitant medication; (6) presence of a serious medical condition; (7) known to interact with study medication; (8) pregnancy or lactation; (9) allergy to any medication in this study; and (10) presence of Zollinger–Ellison syndrome. All participants gave written informed consent. Our study was approved in the ethic committee of Taipei City Hospital (TCHIRB-1011111).

***Interventions***

The participants were randomly assigned to 10-d sequential therapy (rabeprazole, 20 mg and amoxicillin, 1 g twice daily for 5 d, following by rabeprazole 20 mg, clarithromycin 500 mg and metronidazole 500 mg twice daily for 5 d) or 14-d hybrid therapy) therapy (rabeprazole, 20 mg and amoxicillin 1 g for 7 d, following by rabeprazole 20 mg, amoxicillin 1 g, clarithromycin 500 mg and metronidazole 500 mg twice daily for 7 d).

A trained interviewer used a standardized questionnaire to obtain demographic data and medical history. Patients were given a written handout with instructions on how to take the medications correctly.

***Follow-up and outcomes***

Patients were asked to return 2 wk after the start of drug administration to access drug compliance and adverse events. Drug compliance was accessed via pill counts. Compliance termed as good was defined as taking more than 80% of the total medication or poor by counting unused medication after the treatment was completed. Eradication efficacy was assessed by (1) follow-up endoscopy with rapid urease test and histological examination 8 wk after the end of anti-*H. pylori* therapy; or (2) 13C-urea breath test at least 4 wk after completion of treatment. Eradication was defined as either negative results of both urease test and histology or a negative result of the urea breath test.

***Questionnaires***

A complete medical history and demographic data were obtained from each patient, including age, sex, medical history, history of smoking, and alcohol, coffee and tea consumption. Smokers were defined as those who consumed more than 1 pack of cigarettes a week, and drinkers were those who drank more than 1 cup of alcoholic beverage per day. The adverse events evaluated including diarrhea, constipation, anorexia, nausea, vomiting, abdominal pain, skin rash, headache, dizziness, bitter taste and fatigue. We accessed adverse events according to a 4-point scale system: none; mild (discomfort annoying but not interfering with daily life); moderate (discomfort resulting in interference with daily life); severe (discomfort resulting in discontinuation of eradication drug)

***Rapid urease test and 13C-urea test***

The results of rapid urease test (Delta West Bently, Western, Australia) were interpreted as positive if the color of the gel turned pink or red 6 h after examination at room temperature. The 13C-urea was manufactured by the Wagner Analysen Technik Vertriebs GmbH, Germany. 75 mg 13C-Urea mixed with 100 ml water was used as the test drink. The staffs who were blind to the *H. pylori* status performed the tests.

***Culture and pathological examination***

Gastric specimens were taken, one specimen from the lesser curvature site of the antrum and another from the lesser curvature site of corpus for histological examination. The specimens were fixed with formalin, embedded in paraffin, and stained with hematoxylin and erosin. The results form Gram stain was considered positive when a curvy, gram-negative bacterium was found. The histological features of the gastric mucosa were graded according to the updated Sydney System[24]. The histopathologists were blinded to patient status and the results of other laboratory test.

Biopsy specimens were rubbed on the surface of a Columbia blood agar plate and then incubated at 35 ℃ under microaerobic conditions for 4-5 d. Culture of *H. pylori* was considered positive if 1 or more colonies showed gram-negativity, oxidase (+), catalase(+), urease(+), and spiral or curved rods in morphology.

***Antimicrobial resistance***

One antral gastric biopsy specimen was obtained for isolation of *H. pylori*. *H. pylori* subculturing was done by rubbing the specimens on the surface of a Campy-BAP agar plate (Brucella agar; Difco, Sparks, Maryland) + IsoVitalex (Gibco, Grand Island, New York) + 10% whole sheep blood) followed by incubation at 37 ℃under microaerobic conditions (5% O2, 10% CO2, and 85% N2) for 4-5 d. *H. pylori* strains were tested for clarithromycin, tetracycline, metronidazole, amoxicillin, and levofloxacin, susceptibility using E-test (AB Biodisck, Solna, Sweden)*. H. pylori* strains with a minimal inhibitory concentration (MIC) value > 1 µg/mL, > 4 µg/mL, > 8 µg/mL, > 0.5 µg/mL, and > 1 µg/mL were defined as resistance to clarithromycin, tetracycline, metronidazole, amoxicillin, and levofloxacin respectively.

***Statistical analysis***

The variables of primary outcome were the rates of eradication, adverse events and compliance. The difference of patients’ age in two groups was examined using student *t*-test. A two-sided *P* value of less than 0.05 was considered statistically significant. The distribution of gender, smoking, alcohol consumption, coffee ingestion, NSAID user and initial endoscopic diagnosis between subjects in sequential and hybrid groups were compared by **2 test. The same method was applied to compare the treatment efficacy and the frequency of side effects of the two regimens. The data were analyzed using the IBM SPSS statistics (version 22; SPSS Inc., Chicago, IL, United States).

Eradication rates were examined by ITT and PP analyses. ITT analysis included all randomly assigned patients who had taken at least one dose of medication. Patients with unknown *H. pylori* infection status after treatment were considered treatment failures for the purposes of ITT analyses. Patients with unknown *H. pylori* infection status or with major protocol violations were excluded for the PP analyses. A two-sided *P* value of less than 0.05 was considered statistically significant.

**Results**

***Characteristics of the study groups***

A total of 175 patients with *H. pylori*-infection were randomly assigned to sequential (*n =* 87) or hybrid (*n =* 88) therapies. The first patient was randomized on March 18, 2013 and the last ended treatment on May 1, 2014. All subjects were included in the ITT analysis for *H. pylori* eradication. The baseline demographic and clinical characteristics of patients at entry are summarized in Table 1. The two groups had comparable age, history of smoking, alcohol consumption, ingestion of coffee, NSAID user, underlying diseases, endoscopic finding and *H. pylori* density. Among the subjects, four with poor compliance and four lost to follow-up were excluded from PP analysis for *H. pylori* eradication. Figure 1 summarizes the patient disposition.

***Outcome of sequential and hybrid therapies***

The therapeutic outcomes are shown in Table 2. PP analysis demonstrated that hybrid therapy achieves a better eradication rate than sequential therapy [sequential, 81.9% (68/83), 95%CI: 73.6%-90.2% *vs* hybrid, 96.4% (81/84), 95%CI: 92.5%-100.4%, *p =* 0.01). For ITT analysis, the result was similar (sequential, 78.2% (68/87), 95%CI: 69.5%-86.8% *vs* hybrid, 92% (81/88), 95%CI: 86.4%-97.7%, *p =* 0.01).

Both groups displayed good compliance rates (sequential, 97.6% *vs* hybrid, 96.5%; *P =* 0.99). The results were the same when compliance was defined as taking > 80% of the study medications.

***Adverse events***

Adverse events were reported in 112 (64%) of the 175 patients (Table 3); 69% (60/87) of the sequential group and 59% (52/88) of the hybrid group report at least one adverse event during eradication therapy. The frequency was similar between the two groups (*P =* 0.164). Bitter taste (40%) and diarrhea (18.9%) were the most common adverse events. Four patients discontinued the treatments owing to adverse events (< 80% of total medicine). Two patients (2.3%) were from sequential groups (diarrhea, one patients; headache, one patient). Two patients (2.4%) were from hybrid group (nausea, one patient; headache, one patient). Overall, four patients had poor drug compliance.

***Antibiotic resistance***

*H. pylori* strain was successfully isolated from 124 (70.9%) of all enrolled patients who underwent bacterial culture during the initial endoscopy. The rates of resistance were as follows: amoxicillin, 0% (0/124); metronidazole, 37.9% (47/124); clarithromycin, 15.3% (19/124); tetracycline, 0.8% (1/124); levofloxacin, 16.9% (21/124). The dual antibiotics resistant rate (metronidazole and clarithromycin) was 8.9% (11/124).

***Factors influencing efficacy of eradication therapy***

The clinical and bacterial factors influencing the efficacy of therapy are shown in Table 4. Univariate analysis for the clinical and bacterial factors did not identify any risk factors associated with treatment failure. Those with single, dual (metronidazole and clarithromycin) antibiotics resistance were not a predictor of eradication in both groups. Compliance, smoking and alcohol consumption did not affect the result in either group.

**Discussion**

The standard triple therapy is the most used treatment in routine clinical practice. However, the raising prevalence of clarithromycin and metronidazole resistance in recent year, has caused a corresponding decrease in the eradication rates of *H. pylori* infection. It is clear that alternative regimens, particular for patients with clarithromycin-resistant strains of *H. pylori*, are urgently needed. We conducted a randomized, controlled trial to access sequential and hybrid therapies for *H. pylori* infection. The finding of this studydemonstrated a significantly higher eradication rate of hybrid therapy than sequential therapy, whether using ITT (92% *vs* 78.2%) or PP (96.4% *vs* 81.9%) analysis.

The reported eradication rate of sequential therapy varies between 78% and 97%[[18](#_ENREF_18)]. Most of the studies conducted in Italy showed that the sequential therapy was more effective than triple therapy. Conversely, the studies from Latin America and South Korea reported sequential therapy was not superior to triple therapy[25,26]. The difference in the prevalence of antibiotic resistance between the studies was probably the most important explanation. Although Zullo *et al*[27] found the squential therapy was not affected by metronidazole and clarithromycin resistance, except in the presence of dual metronidazole and clarithromycin resistance. Another study from Liou *et al*[28] noted the 10-days sequential therapy was affected by metronidazole or clarithromycin resistance. The eradication rate was 73% (32/44) in metronidazole resistant group and 59% (10/17) in clarithromycin resistant group. The sensitivity test of our study showed the resistance rate of clarithromycin and metronidazole was 15.3% and 37.9% respectively. Ｗe did not find a significant effect of metronidazole and clarithromycin resistance. However, the eradication rate was 66.7% in the presence of metronidazole resistance.

According to Maastricht IV consensus report, bismuth-containing quadruple or sequential therapy should be used empirically in the ﬁrst-line therapy, when the clarithromycin resistance rate is higher than 15%–20%[29]. Nevertheless, our data didn’t lend support to the use of sequential therapy as first-line therapy due to the acceptable eradication rate (81.9%). Taken the latest results into consideration, a more efficient therapy should be needed.

Hsu *et al*[20] reported 99.1% eradication rate with hybrid regimen by PP analysis. They concluded that the high eradication rate of hybrid regimen can be attributed to the treatment duration extending and continuing the amoxicillin through the entire 14 d therapy. In particular, the eradication rate of hybrid therapy for those with dual resistance was 100%. Sardarian *et al*[23] reported an eradication rate of 92.9% with hybrid regimen compared to the 76.7% with the sequential regimen(*p =* 0.001). In our study, hybrid therapy achieved 96.4% eradication rate (grade A success) by PP analysis.

It has previously been suggested that sequential therapy was likely to fail in the presence of dual clarithromycin and metronidazole resistance. A study by Wu *et al*[30] reported three patients in the sequential group had strains of *H. pylori* with dual resistance, and the eradication rate was 33.3% (1/3). Another study from Vaira *et al*[31] reported none of the 4 patients with dual resistance strains achieved eradication with the sequential treatment. Our data showed sequential therapy achieved 71.4% (5/7) eradication rate in patients with dual resistance strains. On the other hand, hybrid therapy achieved 100% (4/4) eradication rate and were consistent with the study report from Hsu et al. These arguments are based on the results from a very small subset of patients in a single study and, therefore, more studies are needed.

Smoking had been showed to reduce the effectiveness of treatment and appeared possibly important in some sequential trials[32-35]. In our study, there’s no significant effect of smoking in both groups. Even so, the prevalence of smoking was relatively low in our study population. Our study also demonstrated that the eradication rate was not affected by bacterial density, alcohol consumption, compliance, gastroduodenal disease and antibiotic resistance in the both groups.

In this study, both regimens were well tolerated and exhibited good compliance. Fifty-nine percent of hybrid group and 69% of sequential group reported at least one adverse event during the eradication therapy. The frequency was similar between the two groups (*p =* 0.22). Most adverse events were mild, only four patients (hybrid group, two patients; sequential group, two patients) discontinued therapy due to severe adverse events. The most common adverse event was bitter taste in both groups; 31 patients (36%) in the hybrid and 39 patients (45.9%) in the sequential group. But it did not result in any therapy discontinuation. Sardarian *et al*[23] compared the adverse events between hybrid therapy and sequential therapy, they also reported a similar side effect with bad taste having the most common incidence.

Both the hybrid and sequential therapy had similar patient compliance in our study (97.6% *vs* 97.5%, *p =* 0.99). This is in accordance with other study reports. Sardarian *et al*[23] reported 96.7% adherence in the hybrid therapy group and 98.6% in the sequential therapy group. The relative complexity of the hybrid regimen did not affect the compliance.

Some limitations acknowledged in our study. First, this is a single-center study comprising a relatively small sample size. A large, multicenter, randomized study is probably necessary to confirm the efficacy of those therapies. Second, antibiotic susceptibility data are available in only 70.9% of patients, which might raise the possibility of selection bias. This percentage was mainly related to the fact that the culture rate of *H. pylori* is less than perfect[36].

Thus, in conclusion, due to a grade A (> 95%) successful rate for *H. pylori* eradication, similar compliance and side events, hybrid therapy seems to be an appropriate eradication regimen in Taiwan.

**comments**

***Background***

The *Helicobacter pylori* (*H. pylori*) infection causes chronic gastritis which significantly increases the risk of developing peptic ulcers and gastric malignancy. The failure rates of standard triple therapies are increasing. Sequential therapy and hybrid have a better eradication rate of *H. pylori* infection than standard triple therapy. Ｈowever, little data exist on the comparison between sequential therapy and hybrid therapy.

***Research frontiers***

Sequential regimen and hybrid regimen for the treatment of *H. pylori* infection were compared.

***Innovations and breakthroughs***

In this study, a head-to-head comparison between hybrid therapy and sequential therapy was performed. Bacterial eradication was achieved in 92% and 78.2% at intension-to-treat, respectively, and 96.4% and 81.9% at per-protocol analysis, respectively, with comparable prevalence of side events, and comparable compliance to the therapy.

***Applications***

The results in this paper suggest that hybrid therapy seems to be an appropriate eradication regimen than sequential therapy. Larger studies and studies in different regions are needed to confirm this finding.

***Terminology***

Hybrid therapy starts with proton pump inhibitor (PPI) plus amoxicillin for 7 d, and follow by a quadruple regimen of a PPI, amoxicillin, clarithromycin, and metronidazole for the next 7 d. Sequential therapy starts with PPI plus amoxicillin for 5 d, followed by a triple regimen of a PPI, clarithromycin, and metronidazole for the next 5 d.

***Peer- review***

This is an interesting paper that compares the efficacy of sequential and hybrid therapy for *H. pylori* infection in Taiwan. The authors should indicate in the abstract that the treatment comparisons were carried out on patients that had not been treated for *H. pylori* before. Also in the conclusion of the abstract and the main body of the paper, indicate that the “grade A (> 95%) success rate for *H. pylori* eradication” was by per protocol analysis.

**References**

1 **Vaira D**, Miglioli M, Mulè P, Holton J, Menegatti M, Vergura M, Biasco G, Conte R, Logan RP, Barbara L. Prevalence of peptic ulcer in Helicobacter pylori positive blood donors. *Gut* 1994; **35**: 309-312 [PMID: 8150337 DOI: 10.1136/gut.35.3.309]

2 **Lin JT**, Wang JT, Wang TH, Wu MS, Lee TK, Chen CJ. Helicobacter pylori infection in a randomly selected population, healthy volunteers, and patients with gastric ulcer and gastric adenocarcinoma. A seroprevalence study in Taiwan. *Scand J Gastroenterol* 1993; **28**: 1067-1072 [PMID: 8303209 DOI: 10.3109/00365529309098311]

3 **Marshall BJ**. Helicobacter pylori. *Am J Gastroenterol* 1994; **89**: S116-S128 [PMID: 8048402]

4 **Rauws EA**, Tytgat GN. Cure of duodenal ulcer associated with eradication of Helicobacter pylori. *Lancet* 1990; **335**: 1233-1235 [PMID: 1971318 DOI: 10.1016/0140-6736(90)91301-P]

5 **Asghar RJ**, Parsonnet J. Helicobacter pylori and risk for gastric adenocarcinoma. *Semin Gastrointest Dis* 2001; **12**: 203-208 [PMID: 11478753]

6 **Sipponen P**. Gastric cancer: pathogenesis, risks, and prevention. *J Gastroenterol* 2002; **37** Suppl 13: 39-44 [PMID: 12109664 DOI: 10.1007/BF02990098]

7 **Gillen D**, McColl KE. Gastroduodenal disease, Helicobacter pylori, and genetic polymorphisms. *Clin Gastroenterol Hepatol* 2005; **3**: 1180-1186 [PMID: 16361041 DOI: 10.1016/S1542-3565(05)00896-7]

8 **Ernst PB**, Peura DA, Crowe SE. The translation of Helicobacter pylori basic research to patient care. *Gastroenterology* 2006; **130**: 188-206; quiz 212-3 [PMID: 16401482 DOI: 10.1053/j.gastro.2005.06.032]

9 **Uemura N**, Okamoto S. Effect of Helicobacter pylori eradication on subsequent development of cancer after endoscopic resection of early gastric cancer in Japan. *Gastroenterol Clin North Am* 2000; **29**: 819-827 [PMID: 11190066 DOI: 10.1016/S0889-8553(05)70149-7]

10 . II United European Gastroenterology Week. Barcelona, Spain, July 19-24, 1993. Abstracts. *Gut* 1993; **34**: S1-49 [PMID: 8102109]

11 **Malfertheiner P**, Megraud F, O'Morain C, Bazzoli F, El-Omar E, Graham D, Hunt R, Rokkas T, Vakil N, Kuipers EJ. Current concepts in the management of Helicobacter pylori infection: the Maastricht III Consensus Report. *Gut* 2007; **56**: 772-781 [PMID: 17170018 DOI: 10.1136/gut.2006.101634]

12 **Graham DY**, Fischbach L. Helicobacter pylori treatment in the era of increasing antibiotic resistance. *Gut* 2010; **59**: 1143-1153 [PMID: 20525969 DOI: 10.1136/gut.2009.192757]

13 **Egan BJ**, Marzio L, O'Connor H, O'Morain C. Treatment of Helicobacter pylori infection. *Helicobacter* 2008; **13** Suppl 1: 35-40 [PMID: 18783520 DOI: 10.1111/j.1523-5378.2008.00639.x]

14 **Hsu PI**, Lai KH, Lin CK, Chen WC, Yu HC, Cheng JS, Tsay FW, Wu CJ, Lo CC, Tseng HH, Yamaoka Y, Chen JL, Lo GH. A prospective randomized trial of esomeprazole- versus pantoprazole-based triple therapy for Helicobacter pylori eradication. *Am J Gastroenterol* 2005; **100**: 2387-2392 [PMID: 16279889 DOI: 10.1111/j.1572-0241.2005.00264.x]

15 **Wu IC**, Wu DC, Hsu PI, Lu CY, Yu FJ, Wang TE, Chang WH, Chen JJ, Kuo FC, Wu JY, Wang WM, Bair MJ. Rabeprazole- versus esomeprazole-based eradication regimens for H. pylori infection. *Helicobacter* 2007; **12**: 633-637 [PMID: 18001406 DOI: 10.1111/j.1523-5378.2007.00553.x]

16 **Houben MH**, van de Beek D, Hensen EF, de Craen AJ, Rauws EA, Tytgat GN. A systematic review of Helicobacter pylori eradication therapy--the impact of antimicrobial resistance on eradication rates. *Aliment Pharmacol Ther* 1999; **13**: 1047-1055 [PMID: 10468680 DOI: 10.1046/j.1365-2036.1999.00555.x]

17 **Padol S**, Yuan Y, Thabane M, Padol IT, Hunt RH. The effect of CYP2C19 polymorphisms on H. pylori eradication rate in dual and triple first-line PPI therapies: a meta-analysis. *Am J Gastroenterol* 2006; **101**: 1467-1475 [PMID: 16863547 DOI: 10.1111/j.1572-0241.2006.00717.x]

18 **Gisbert JP**, Calvet X, O'Connor A, Mégraud F, O'Morain CA. Sequential therapy for Helicobacter pylori eradication: a critical review. *J Clin Gastroenterol* 2010; **44**: 313-325 [PMID: 20054285 DOI: 10.1097/mcg.0b013e3181c8a1a3]

19 **Gatta L**, Vakil N, Leandro G, Di Mario F, Vaira D. Sequential therapy or triple therapy for Helicobacter pylori infection: systematic review and meta-analysis of randomized controlled trials in adults and children. *Am J Gastroenterol* 2009; **104**: 3069-379; quiz 1080 [PMID: 19844205 DOI: 10.1038/ajg.2009.555]

20 **Hsu PI**, Wu DC, Wu JY, Graham DY. 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 [PMID: 21435092 DOI: 10.1111/j.1523-5378.2011.00828.x]

21 **Graham DY**, Lu H, Yamaoka Y. A report card to grade Helicobacter pylori therapy. *Helicobacter* 2007; **12**: 275-278 [PMID: 17669098 DOI: 10.1111/j.1523-5378.2007.00518.x]

22 **Zullo A**, Scaccianoce G, De Francesco V, Ruggiero V, D'Ambrosio P, Castorani L, Bonfrate L, Vannella L, Hassan C, Portincasa P. Concomitant, sequential, and hybrid therapy for H. pylori eradication: a pilot study. *Clin Res Hepatol Gastroenterol* 2013; **37**: 647-650 [PMID: 23747131 DOI: 10.1016/j.clinre.2013.04.003]

23 **Sardarian H**, Fakheri H, Hosseini V, Taghvaei T, Maleki I, Mokhtare M. Comparison of hybrid and sequential therapies for Helicobacter pylori eradication in Iran: a prospective randomized trial. *Helicobacter* 2013; **18**: 129-134 [PMID: 23121338 DOI: 10.1111/hel.12017]

24 **Stolte M**, Meining A. The updated Sydney system: classification and grading of gastritis as the basis of diagnosis and treatment. *Can J Gastroenterol* 2001; **15**: 591-598 [PMID: 11573102]

25 **Greenberg ER**, Anderson GL, Morgan DR, Torres J, Chey WD, Bravo LE, Dominguez RL, Ferreccio C, Herrero R, Lazcano-Ponce EC, Meza-Montenegro MM, Peña R, Peña EM, Salazar-Martínez E, Correa P, Martínez ME, Valdivieso M, Goodman GE, Crowley JJ, Baker LH. 14-day triple, 5-day concomitant, and 10-day sequential therapies for Helicobacter pylori infection in seven Latin American sites: a randomised trial. *Lancet* 2011; **378**: 507-514 [PMID: 21777974 DOI: 10.1016/S0140-6736(11)60825-8]

26 **Kim YS**, Kim SJ, Yoon JH, Suk KT, Kim JB, Kim DJ, Kim DY, Min HJ, Park SH, Shin WG, Kim KH, Kim HY, Baik GH. Randomised clinical trial: the efficacy of a 10-day sequential therapy *vs* a 14-day standard proton pump inhibitor-based triple therapy for Helicobacter pylori in Korea. *Aliment Pharmacol Ther* 2011; **34**: 1098-1105 [PMID: 21923713 DOI: 10.1111/j.1365-2036.2011.04843.x]

27 **Zullo A**, De Francesco V, Hassan C, Morini S, Vaira D. The sequential therapy regimen for Helicobacter pylori eradication: a pooled-data analysis. *Gut* 2007; **56**: 1353-1357 [PMID: 17566020 DOI: 10.1136/gut.2007.125658]

28 **Liou JM**, Chen CC, Chen MJ, Chen CC, Chang CY, Fang YJ, Lee JY, Hsu SJ, Luo JC, Chang WH, Hsu YC, Tseng CH, Tseng PH, Wang HP, Yang UC, Shun CT, Lin JT, Lee YC, Wu MS. Sequential versus triple therapy for the first-line treatment of Helicobacter pylori: a multicentre, open-label, randomised trial. *Lancet* 2013; **381**: 205-213 [PMID: 23158886 DOI: 10.1016/S0140-6736(12)61579-7]

29 **Malfertheiner P**, Megraud F, O'Morain CA, Atherton J, Axon AT, Bazzoli F, Gensini GF, Gisbert JP, Graham DY, Rokkas T, El-Omar EM, Kuipers EJ. Management of Helicobacter pylori infection--the Maastricht IV/ Florence Consensus Report. *Gut* 2012; **61**: 646-664 [PMID: 22491499 DOI: 10.1136/gutjnl-2012-302084]

30 **Wu DC**, Hsu PI, Wu JY, Opekun AR, Kuo CH, Wu IC, Wang SS, Chen A, Hung WC, Graham DY. Sequential and concomitant therapy with four drugs is equally effective for eradication of H pylori infection. *Clin Gastroenterol Hepatol* 2010; **8**: 36-41.e1 [PMID: 19804842 DOI: 10.1016/j.cgh.2009.09.030]

31 **Vaira D**, Zullo A, Vakil N, Gatta L, Ricci C, Perna F, Hassan C, Bernabucci V, Tampieri A, Morini S. Sequential therapy versus standard triple-drug therapy for Helicobacter pylori eradication: a randomized trial. *Ann Intern Med* 2007; **146**: 556-563 [PMID: 17438314 DOI: 10.7326/0003-4819-146-8-200704170-00006]

32 **Treiber G**, Wittig J, Ammon S, Walker S, van Doorn LJ, Klotz U. Clinical outcome and influencing factors of a new short-term quadruple therapy for Helicobacter pylori eradication: a randomized controlled trial (MACLOR study). *Arch Intern Med* 2002; **162**: 153-160 [PMID: 11802748 DOI: 10.1001/archinte.162.2.153]

33 **Moayyedi P**. Sequential regimens for Helicobacter pylori eradication. *Lancet* 2007; **370**: 1010-1012 [PMID: 17889226 DOI: 10.1016/S0140-6736(07)61455-X]

34 **Suzuki T**, Matsuo K, Ito H, Sawaki A, Hirose K, Wakai K, Sato S, Nakamura T, Yamao K, Ueda R, Tajima K. Smoking increases the treatment failure for Helicobacter pylori eradication. *Am J Med* 2006; **119**: 217-224 [PMID: 16490464 DOI: 10.1016/j.amjmed.2005.10.003]

35 **Broutet N**, Tchamgoué S, Pereira E, Lamouliatte H, Salamon R, Mégraud F. Risk factors for failure of Helicobacter pylori therapy--results of an individual data analysis of 2751 patients. *Aliment Pharmacol Ther* 2003; **17**: 99-109 [PMID: 12492738 DOI: 10.1046/j.1365-2036.2003.01396.x]

36 **Ricci C**, Holton J, Vaira D. Diagnosis of Helicobacter pylori: invasive and non-invasive tests. *Best Pract Res Clin Gastroenterol* 2007; **21**: 299-313 [PMID: 17382278 DOI: 10.1016/j.bpg.2006.11.002]

**P-Reviewer:** Engin AB, Smith SM **S-Editor:** Ma yj **L-Editor:** **E-Editor:**

**Table 1 Demographic distribution of the subjects receiving different eradication regimens**

|  |  |  |  |
| --- | --- | --- | --- |
| **Characteristics** | **Hybrid therapy (*n =* 88)** | **Sequential therapy (*n =* 87)** | ***P*-value** |
| Age (mean ± SD), yr  | 52.1 ± 13.0 | 54.6 ± 13.2 | 0.26 |
| Gender (male/female) | 39/49 | 25/62 | 0.04 |
| Smoking | 14 | 6 | 0.10 |
| Alcohol consumption  | 20 | 18 | 0.74 |
| Ingestion of coffee | 61 | 57 | 0.59 |
| NSAID user | 8 | 8 | 0.96 |
| Underlying diseases | 26 | 23 | 0.77 |
| Endoscopic finding(Peptic ulcer/non-ulcer dyspepsia) | 46/42 | 32/55 | 0.06 |
| *Helicobacter pylori* density (mild/moderate/marked) | 8/42/38 | 4/57/26 | 0.26 |

|  |
| --- |
| **Exclusion** |
| No compliance (*n* = 2)Drug interruption (*n* = 2) |

|  |
| --- |
| **Exclusion** |
| No compliance (*n* = 2)Drug interruption (*n* = 2) |

Included in PP, *n* = 84

Included in PP, *n* = 83

Randomized, *n* = 175

Included in ITT, *n* = 88

Included in ITT, *n* = 87

Hybrid group, *n* = 88

Sequential group, *n* = 87

**Figure 1 Flow chart of method of follow up and treatment efficacy.** ITT: Intension-to-treat; PP: Per-protocol.

**Table 2 outcomes of sequential and hybrid therapy**

|  |  |  |  |
| --- | --- | --- | --- |
| **Variable** | **Hybrid therapy** | **Sequential therapy** |  |
| **Patients** | **Eradication Rate** | **95% confidence interval** | **Patients** | **Eradication Rate** | **95% confidence interval** | ***P*-value** |
| Intension-to-treat analysis | 88 | 92% | 86.4-97.7 | 87 | 78.2% | 69.5-86.8 | 0.01 |
| Per-protocol analysis | 84 | 96.4% | 92.5-100.4 | 83 | 81.9% | 73.6-90.2 | 0.01 |

**Table 3 Adverse events during treatment**

|  |  |  |  |
| --- | --- | --- | --- |
|  | **Hybrid therapy** | **Sequential therapy**  | ***P*-value** |
| Compliance, *n* (%) | 84 (97.7) | 83 (97.6) | 0.99 |
| Side effect, *n* (%) | 52 (59) | 60 (69) | 0.22 |
| Diarrhea | 16/1/11 | 15/0/0 | 0.42 |
| Constipation | 1/0/0 | 2/0/0 | 0.50 |
| Anorexia | 0 | 1/0/0 | 0.70 |
| Nausea | 4/1/0 | 9/0/1 | 0.92 |
| Vomiting | 5/2/0 | 3/1/0 | 0.57 |
| Abdominal pain | 5/0/0 | 9/0/0 | 0.87 |
| Skin rash | 3/2/0 | 1/2/0 | 0.74 |
| Headache | 9/1/1 | 16/1/1 | 0.41 |
| Dizziness | 6/1/0 | 9/0/0 | 0.70 |
| Bitter taste | 25/6/0 | 36/3/0 | 0.17 |
| Fatigue | 3/0/0 | 4/0/0 | 0.30 |

1Numbers of patients who suffered from, mild, moderate, and severe adverse events.

**Table 4 Univariate analysis of the clinical factors influencing the efficacy of eradication therapy *n* (%)**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Eradication rate** | **Sequential therapy** | ***P*-value** | **Hybrid therapy**  | ***P*-value** |
| Resistance (*n =* 124) | *n =* 59 |  | *n =* 65 |  |
| Metronidazole |  | 0.22 |  | 0.74 |
| Susceptible | 32 (84.2)  |  | 37 (94.9)  |  |
| Resistant | 14 (66.7)  |  | 25 (96.1) |  |
| Clarithromycin |  | 0.73 |  | 0.51 |
| Susceptible | 37 (77.1)  |  | 54 (94.7)  |  |
| Resistant | 9 (81.8)  |  | 8 (100)  |  |
| Tetracycline |  | 0.59 |  | NA |
| Susceptible | 45 (77.6)  |  | 62 (95.4)  |  |
| Resistant | 1 (100)  |  |  |  |
| Levofloxacin |  | 0.46 |  | 0.45 |
| Susceptible | 36 (75.0)  |  | 52 (94.5) |  |
| Resistant | 10 (90.9)  |  | 10 (100) |  |
| Amoxicillin |  | NA |  | NA |
| Susceptible | 46 (78.0)  |  | 62 (95.4) |  |
| Resistant |  |  |  |  |
| Dual resistance |  | 0.66 |  | 0.65 |
| Present | 5 (71.4) |  | 4 (100) |  |
| Absent | 41 (78.8) |  | 58 (95) |  |
| Compliance |  | 0.47 |  | 0.66 |
| Good | 66 (79.5) |  | 78 (94.0) |  |
| Poor | 2 (100) |  | 3 (100) |  |
| Smoking |  | 0.20 |  | 0.31 |
| Present | 6 (100) |  | 14 (100) |  |
| Absent | 62 (78.5) |  | 67 (93.1) |  |
| Alcohol |  | 0.29 |  | 0.21 |
| Present | 16 (88.9) |  | 20 (100) |  |
| Absent | 52 (77.6) |  | 61 (92.4) |  |
| Gastroduodenal disease |  | 0.91 |  | 0.57 |
| Non-ulcer dyspepsia | 43 (79.6) |  | 38 (92.7) |  |
| Peptic ulcer | 25 (80.6) |  | 43 (95.6) |  |
| Bacterial density |  | 0.31 |  | 0.40 |
| Mild | 4 (100) |  | 7 (87.5) |  |
| Moderate/marked | 64 (79) |  | 74 (94.9)  |  |