Original Article

The effects of sequential treatment as a first-line therapy for *Helicobacter pylori* eradication

Mehmet DEMİR, Hilmi ATASEVEN

Aim: There is no effective eradication regimen for *Helicobacter pylori* (HP) in Turkey. Recent studies have shown that sequential therapy may be superior to the standard triple therapy in terms of the eradication of HP. In this study, we aim to assess the efficacy of a 14-day sequential treatment regimen as a first-line therapy for HP eradication.

Materials and methods: This is a prospective, open-label, single-centre study. The study involved 86 consecutive patients with nonulcer dyspepsia. All patients were randomly assigned into 2 study groups at a 2:1 ratio using random sampling numbers. The first group of patients were administered a sequential treatment: pantoprazole 2 × 40 mg and amoxicillin 2 × 1000 mg for the first 7 days and pantoprazole 2 × 40 mg, metronidazole 2 × 500 mg, and tetracycline 4 × 500 mg for the remaining 7 days. The second group of patients were administered pantoprazole 2 × 40 mg, amoxicillin 2 × 1000 mg, and clarithromycin 2 × 500 mg (PAC) for 14 days. Eradication was defined as the absence HP as assessed with the (14-C) urea breath test 4 weeks after the end of the antimicrobial therapy.

Results: The eradication rate in the sequential group was 56.1% for the ITT analysis and 57.1% for the PP analysis, the eradication rate of the PAC group was 58.6 % for both PP and ITT analysis. There was a statistically significant difference between the eradication rates of the groups for both PP and ITT analysis. There was no statistically significant difference in the adverse effects encountered in both groups (10.5% versus 13.8% P > 0.05).

Conclusion: These results suggest that a 14-day sequential eradication regimen is not effective as a first-line therapy for HP eradication.

Key words: Sequential treatment; helicobacter pylori eradication

Helicobacter pylori eradikasyonu için birinci basamak tedavi olarak ardışık tedavi rejiminin etkinliği

Amaç: Ülkemizde *Helicobacter pylori* (HP) için etkili eradikasyon rejimi yoktur. Son dönemlerdeki bilgiler HP eradikasyonu için ardışık tedavi rejimininin standart üçlü tedaviden üstün olduğunu göstermiştir. Bizim bu çalışmadaki amacımız HP eradikasyonu için birinci basamak tedavi olarak 14 günlük ardışık tedavi rejiminin etkinliğini araştırmaktı.

Yöntem ve gereç: Bu çalışma prospektif, open-label, tek merkezli bir çalışmadır. Çalışmaya ardışık 86 nonülser dispepsili hasta dahil edildi. Tüm hastalar rastgele 2 çalışma grubuna 2:1 oranında rastgele örnekleme sayıları kullanarak ayrıldı. İlk gruptaki hastalara ardışık tedavi verildi; pantoprazol 2 × 40 mg ve amoksisiklin 2 × 1000 mg ilk 7 gün, ve pantoprazol 2 × 40 mg, metronidazol 2 × 500 mg, tetrasiklin 4 × 500 mg geriye kalan 7 gün. İkinci gruptaki hastalara pantoprazol 2 × 40 mg ve amoksisiklin 2 × 1000 mg ve klaritromisin 2 × 500 mg (PAC) verildi.

Bulgular: Ardışık gurupta eradikasyon oranı ITT analizi ile % 56,1 ve PP analizi ile %,57,1, PAC grupta hem ITT hem de PP analizi ile % 58,6 idi, ve gruplar arasında hem ITT hem de PP analizi ile istatistiksel olarak anlamlı fark yoktu. Gruplar arasında yan etki açısından istatistiksel olarak anlamlı fark yoktu (% 10,5 karşın % 13,8 P > 0,05).

Correspondence: *Mehmet DEMİR, Department of Gastroenterology, Erzurum Region Education and Research Hospital, Erzurum - TURKEY*
E-mail:drmehmetdemir@yahoo.com

Sonuç: Bu sonuçlar HP eradikasyonu için birinci basamak tedavi olarak 14 günlük ardışık tedavi rejiminin etkin olmadığını düşündürmektedir.

Anahtar sözcükler: Ardışık tedavi, *Helicobacter pylori* eradikasyonu

Introduction

Helicobacter pylori (HP) is a major cause of illness and death worldwide. HP plays an important role in the pathogenesis of chronic gastritis, peptic ulcer disease, gastric mucosa-associated lymphoid tissue lymphoma, and gastric adenocarcinoma (1). The eradication of HP is associated with a decrease in the recurrence of peptic ulcer and its complications and regression of low-grade gastric lymphoma in most cases (2,3). According to Maastricht Consensus reports issued in 2007, the preferred treatment regimens were triple treatments including a proton-pump inhibitor (PPI) or ranitidine bismuth citrate (RBC) plus 2 antibiotics (amoxicillin, metronidazole, or clarithromycin) (4). In Turkey, however, these treatment regimens only provide eradication in approximately 50% of the cases of HP infection (5-8). There is no effective eradication regimen for HP in our country. Therefore, further therapeutic approaches are sought for HP infection. Recent data indicated that sequential therapy may be superior to standard triple therapy for eradication (9-11). Unfortunately, there are limited available data about sequential therapy as a first-line therapy for HP eradication in Turkey and the data that are available are often contradictory (12,13). In the present study, we aimed to assess the efficacy of a 14-day sequential treatment regimen (pantoprazole 2 × 40 mg and amoxicillin 2 × 1000 mg for the first 7 days followed by pantoprazole 2 × 40 mg, tetracycline 4 × 500 mg, and metronidazole 2 × 500 mg for the remaining 7 days) as a first-line therapy for HP eradication.

Materials and methods

This was a prospective, randomized, single-centre study. The study involved 86 consecutive patients with nonulcer dyspepsia admitted to the Regional Education and Research Hospital in Erzurum, Turkey, between September 2008 and May 2009.

Exclusion criteria for all study subjects included prior HP eradication therapy; use of medication including proton pump inhibitors, H-2 receptor blockers, bismuth preparations, prokinetics, non-steroidal anti-inflammatory drugs (NSAIDs), or antibiotics in the preceding 2 months; alcohol addiction; history of gastric surgery or cholecystectomy; decompensated congestive heart failure; liver cirrhosis; chronic renal failure requiring dialysis; diagnosis of malignancy; pregnancy; and active infection requiring antibiotherapy.

Diagnosis of HP infection

All patients included in this study underwent upper gastrointestinal endoscopy (Fujinon EG 450WR5, Japan). In each patient, 2 biopsy specimens from the antrum and 2 from the corpus were taken for pathological examinations, and 1 biopsy specimen was taken from the antrum for a urease test. The presence of HP was identified by its curved-shape morphology, location on the epithelial cell surface (within the gastric pits or in the overlying mucus layer), and positive staining by Giemsa stain. HP status was evaluated by a rapid urease test (RUT) and histology by Giemsa stain. Patients with positive HP results on both histopathology and RUT were included in the study. Of the 153 patients, 86 patients were found to be HP-positive by both RUT and histological examination. Of the remaining 67 patients, 1 patient was found to be HP-positive histologically and negative by RUT, 4 patients were declared positive by RUT and negative by histologic examination, and 62 patients were determined to be negative by both RUT and histologic examination and were thus excluded from the study.

Treatment regimen

All patients were randomly assigned into 1 of the 2 groups at a 2:1 ratio using random sampling numbers. The first group of patients was administered a sequential treatment: pantoprazole 2 × 40 mg and amoxicillin 2 × 1000 mg for the first 7 days and pantoprazole 2 × 40 mg, metronidazole 2 × 500 mg, and tetracycline 4 × 500 mg for the remaining 7 days. The second group of patients was

administered pantoprazole 2 × 40 mg, amoxicillin 2 × 1000 mg, and clarithromycin 2 × 500 mg (PAC) for 14 days. Eradication was defined as the absence HP as assessed with the (14-C) urea breath test 4 weeks after the end of the antimicrobial therapy.

Compliance and safety

Compliance and the frequency of side effects were assessed using a diary completed by the patient during treatment. At the end of the first week and the second week, side-effects of the treatment were evaluated. All adverse events were recorded, irrespective of the relationship to the study medication as assessed by the physician. Compliance with therapy was assessed through return pill count for each patient.

Ethics and consent

The study protocol was carried out in accordance with the Helsinki Declaration revised in 1989, and each patient provided written informed consent in accordance with the Helsinki Declaration.

Statistical analyses

Data were analyzed using SPSS software (Statistical Package for the Social Sciences, version 13.0, SPSS Inc, Chicago, IL, USA). Results are expressed as means ± standard deviation. Statistically significant differences between groups were assessed using the *t* or chi-square tests. P values below 0.05 were considered statistically significant in all analyses. We calculated the per-protocol (PP) and intention-to-treat (ITT) eradication rates and 95% confidence intervals (CI) for both treatments. For the PP analysis, only patients who took all of their medications for the prescribed duration of the therapy were evaluated. For the ITT analysis, all patients were included, including those non-compliant and those who did not complete the full course of therapy.

Results

Demographic data

After exclusions, the study population consisted of 57 patients in the sequential group (29 females, 28 males; mean age: 39.1 ± 14.5 years) and 29 patients in the PAC group (15 females, 14 males; mean age: 38.9 ± 12.9 years). There was no significant difference between the gender and mean age of the 2 groups (P > 0.05). The current smoking ratio was 49.1% (28/57) in the sequential group and 48.2% (14/29) in the PAC group. There was no significant difference between the current smoking of the 2 groups (P > 0.05). The baseline characteristics according of groups are presented in Table 1.

Patient compliance and side-effects

Drug compliance was at an acceptable rate. Compliance rates among sequential group and PAC group were 94% and 98%, respectively. In the sequential group, side-effects were observed in 6 patients (10.5%). They were abdominal pain or discomfort in 2 patients (3.5%), diarrhea in 2 patients (3.5%), nausea and/or vomiting in 1 patient (1.75%), and bloating in 1 patient (1.75%). In the PAC group, side-effects were observed in 4 patients (13.8%). They were abdominal pain or discomfort in 1 patient (3.45%), diarrhea in 1 patient (3.45%), constipation in 1 patient (3.45%), and headache in 1 patient (3.45%). There was no statistically significant difference between the incidence of side effects among the 2 groups (P = 0.655). One patient in the sequential group could not complete the treatment due to severe nausea and vomiting; 56 out of 57 patients completed the study. None of the patients in the PAC group discontinued treatment because of side-effects and all patients were able to complete the study. The distribution of adverse effects among the groups is presented in Table 2.

Eradication of HP

The eradication rate in the sequential group was 56.1% (32/57) for the ITT analysis and 57.1% (32/56) for the PP analysis; the eradication rate of PAC group was 58.6% for both ITT and PP analysis. The eradication rates were not significantly different between the groups with both ITT and PP analysis. All eradication rates, treatment differences, and 95% confidence limits of the eradication rates and differences are summarized in Table 3.

Discussion

The European *Helicobacter pylori* Study Group guidelines advocate a proton pump inhibitor plus 2 antibiotics (clarithromycin or metronidazole in combination with amoxicillin) as a first-line therapy for HP eradication. However, an epidemiologic analysis of trends over 10 years (1996-2005) in the

Table 1. Demographic and clinical characteristics of the patients.

	Sequential group	PAC group	P value
n	57	29	
Age	39.1 ± 14.5	38.9 ± 12.9	NS
Sex (Male/Female)	28/29	14/15	NS
Current smoking (n) (%)	28 (49.1%)	14 (48.2%)	NS

N: number of patients; NS: nonsignificant; F: females, M: males; PAC: pantoprazole - amoxicillin - clarithromycin

Table 2. Distribution of adverse effects among the sequential group and the PAC group.

	Sequential group n: 57	PAC group n: 29	P value
Adverse events (n) (%)			
Abdominal pain or discomfort	2 (3.5%)	1 (3.45%)	
Diarrhea	2 (3.5%)	1 (3.45%)	
Nausea and/or vomiting	1 (1.75%)	-	
Constipation	-	1 (3.45%)	
Bloating	1 (1.75%)	-	
Headache	-	1 (3.45%)	
Total (n) (%)	6 (10.5%)	4 (13.8%)	NS

N: number of patients; NS: nonsignificant; PAC: pantoprazole - amoxicillin - clarithromycin

Table 3. Rates of *Helicobacter pylori* eradication in 2 different treatment regimens

	Sequential group	PAC group	**P value**
ITT % (95% CI)	32/57 56.1% (43%-68%)	17/29 58.6% (40%-76%)	>0.05
PP % (95% CI)	32/56 57.1% (44%-70%)	17/29 58.6% (40%-76%)	>0.05

n: total number of patients, CI: confidence intervals, PP: per protocol, ITT: Intention-to- treat, PAC: pantoprazole-amoxicillin-clarithromycin

Turkish population reported a gradual of decrease in the of eradication success from 79.4% to 61.1% by administering a PPI, amoxicillin, and clarithromycin combination (4,5). In our study, the eradication rate of HP in the PAC group was 58.6%, which was in accordance with the aforementioned study. Recent data suggest that clarithromycin resistance is a growing problem affecting the eradication rate of the HP. It has been shown in a meta-analysis that the cure rate of the infection is reduced to 55% in patients with clarithromycin resistance (14). The rate of resistance to clarithromycin has increased gradually in Turkey. Recently, a study on Turkish dyspeptic population revealed a clarithromycin resistance of 40.2% using PCR method (15). Analysis of the abstracts of the Turkish Gastroenterology Congress from 2001 to 2006 revealed a significant increase in clarithromycin resistance that paralleled the increase in eradication failure (5). Accordingly, we theorized that the cause of the low eradication rate in the PAC group may be related to the higher prevalence of clarithromycin resistance in this country.

Recent interest has focused on sequential therapy, which consists of 5 or 7 days of treatment with a proton-pump inhibitor and one antibiotic (usually amoxicillin) followed by 5 or 7 days of treatment with the proton-pump inhibitor and 2 other antibiotics (usually clarithromycin and a 5-nitroimidazole) (10-13). The rationale for this more complicated approach is that amoxicillin may weaken bacterial cell walls in the initial phase of treatment, preventing the development of drug efflux channels that inhibit such drugs as clarithromycin from binding to ribosomes. This may help to improve the efficacy of clarithromycin in the second phase of treatment (16). Recent data showed that the sequential treatment regimen achieved a significantly higher eradication rate of HP when compared to the standard PPI-based triple regimen in this small selected population (11,12). In this study, HP eradication rates in the sequential therapy group were 56.1% and 57.1% with ITT and PP analyses, respectively, and the overall eradication rate was not significantly better than that of the triple eradication regimen (58.6% with both ITT and PP analysis) (P > 0.05). The results of previous studies in Turkey are controversial. Uygun

et al. reported that a 14-day sequential treatment regimen was significantly more effective than 14 days of triple treatment (72.6% versus 58% with ITT analysis, 80.1% versus 63% with PP analysis) (12). However, Sezgin et al. reported that eradication rates with a 14-day sequential treatment regimen (pantoprazole 40 mg b.d. plus amoxicillin 1000 mg b.d. for 7 days and pantoprazole 40 mg b.d., metronidazole 500 mg b.d., and tetracycline 500 mg q.d. for the remaining 7 days) were 50% for the ITT analysis and 57% for the PP analysis (13). Our findings were in accordance with those of Sezgin et al. (50% versus 56.1% for the ITT analysis and 57% versus 56.1% for the PP analysis). Therefore, our findings suggest that sequential treatment regimens do not have better eradication rates than standard triple therapies in the first-line eradication of HP.

The sequential eradication regimen is not effective as a first-line therapy for HP eradication and it is not clear why. The cause of the low eradication rate in the PAC group may be related to higher prevalence of clarithromycin resistance in this country, but, in our study, the sequential eradication regimen did not include clarithromycin. Studies performed in Turkey have also shown a high prevalence of metronidazole resistance (50%-60%) (17,18). This factor could be responsible for the ineffective eradication, but in vitro resistance to metronidazole is not always predictive of in vivo resistance (19,20). However, increasing the dosage of metronidazole to 500 mg improves the results of therapy when the doses are administered 3 times per day (21).

Drug compliance in the sequential group was at an acceptable rate. In the previous studies, the prevalence of the reported adverse effects with a sequential regimen was low (7%, 36 of 522 patients), and all of the adverse effects were minor (9). The prevalence of them with a 14-day sequential eradication regimen was nearly 10% and no statistically significant differences were found between the triple eradication regimen and the sequential eradication regimen (12). In the present study, adverse effects were detected in 10.2% of patients in the sequential group and 13.8% in the PAC group, although the difference between groups did not reach statistical significance (P > 0.05). These observations suggest that a 14-day sequential

eradication regimen is tolerable and compliance is good.

The lack of any specific susceptibility testing for antibiotics, the non double-blind nature of the study, and the relatively small number of subjects were the most important limitations of the present study. However, antibiotic susceptibility tests are not routinely performed in the first-line eradication treatment of HP and, therefore, the clinical validity of the study is not affected.

There is no effective treatment that can be offered as a first-line therapy for HP eradication in this country. We recently showed that the bismuth-based quadruple eradication regimen as first-line therapy is safe, tolerable, and achieves a high cure

rate in both patients with diabetes mellitus and non-diabetic patients, 81% and 85%, respectively (22). Higher eradication rate is likely to be achieved by quadruple therapy because of the high prevalence of clarithromycin resistance in our populations. In regions that demonstrate a high prevalence of antibiotic resistance (>15%-20%), quadruple therapy is widely recommended (4). The bismuth-based quadruple therapy can therefore be proposed as a first-line therapy for HP eradication in Turkey.

In conclusion, these results suggest that a 14-day sequential eradication regimen is not effective as a first-line therapy for HP eradication. Further studies with new antibiotic associations are needed for more effective eradication of HP in Turkey.

References

1. Dunn BE, Cohen H, Blaser MJ. *Helicobacter pylori*. Clinical Microbiology Reviews 1997; 10: 720-741.

2. Wong BCY, Lam SK, Lai KC, Hu WH, Ching CK, Ho J et al. Triple therapy for *Helicobacter pylori* eradication is more effective than long-term maintenance antisecretory treatment in the prevention of recurrence of duodenal ulcer: a prospective long-term follow-up study. Aliment Pharmacol Ther 1999; 13: 303-309.

3. Bayerdörffer E, Neubauer A, Rudolph B, Thiede C, Lehn N, Eidt S et al. Regression of primary gastric lymphoma of mucosa-associated lymphoid tissue type after cure of *Helicobacter pylori* infection. MALT Lymphoma Study Group. Lancet 1995; 345: 1591-1594.

4. Malfertheiner P, Megraud F, O'Morain C, Bazzoli F, El-Omar E, Graham D et al. Current concepts in the management of *Helicobacter pylori* infection – The Maastricht III consensus report. Gut 2007; 56: 772-81.

5. Kadayıfcı A, Buyukhatipoglu H, Savas M Cemil, Simsek I. Eradication of *Helicobacter pylori* with triple therapy: An epidemiologic analysis of trends in Turkey over 10 years. Clin Ther 2006; 11: 1960-1966.

6. Uygun A, Kadayıfçı A, Yeşilova Z. Hp eradikasyonunda lansoprazol ve pantoprazol içeren üçlü rejimlerin etkinliğinin karşılaştırılması (Comparison of effectiveness of lansoprazole and pantoprazole based triple therapy in *Helicobacter pylori* eradication). Turk J Gastroenterol 2003; (Suppl): PB.08/33)

7. Güliter S, Keleş H, Özkurt ZN, Cengiz DU, Kolukısa E. Can lansoprazole, amoxicillin and clarithromycin combination still be used as a first-line therapy for eradication of *Helicobacter pylori*? Turk J Gastroenterol 2005; 16: 29-33.

8. Ertaş E, Tüzün A, Özden A. *Helicobacter pylori* pozitif fonksiyonel dispepside 7 ve 14 günlük pantoprazol, klaritromisin, amoksisilin kombinasyon tedavisinin eradikasyon etkinliğinin ve eradikasyonun dispeptik semptomlar üzerine etkilerinin degerlendirilmesi. (Effectiveness of 7 and 14 days pantoprazole, amoxicillin, clarithromycin combination treatments in *Helicobacter pylori* positive functional dyspepsia). Turk J Gastroenterol 2004; (Suppl): PB.08/18.

9. Zullo A, Vaira D, Vakil N, Hassan C, Gatta L, Ricci C et al. High eradication rates of *Helicobacter pylori* with a new sequential treatment. Aliment Pharmacol Ther 2003; 17: 719-26.

10. Jafri NS, Hornung CA, Howden CW. Meta-analysis: sequential therapy appears superior to standard therapy for *Helicobacter pylori* infection in patients naive to treatment. Ann Intern Med 2008; 17: 148: 923-31.

11. Tong JL, Ran ZH, Shen J, Xiao SD. Sequential therapy vs. standard triple therapies for *Helicobacter pylori* infection: a meta-analysis. J Clin Pharm Ther 2009; 34: 41-53.

12. Uygun A, Kadayifci A, Yesilova Z, Safali M, Ilgan S, Karaeren N. Comparison of sequential and standard triple-drug regimen for *Helicobacter pylori* eradication: a 14-day, open-label, randomized, prospective, parallel-arm study in adult patients with nonulcer dyspepsia. Clin Ther 2008; 30: 528-34.

13. Sezgin O, Altintaş E, Nayir E, Uçbilek E. A pilot study evaluating sequential administration of a PPI-amoxicillin followed by a PPI-metronidazole-tetracycline in Turkey. Helicobacter. 2007; 12: 629-32.

14. Dore MP, Leandro G, Realdi G, Sepulveda AR, Graham DY. Effect of pretreatment antibiotic resistance to metronidazole and clarithromycin on outcome of Helicobacter pylori therapy: a meta-analytical approach. Dig Dis Sci 2000; 45: 68-76.

15. Sezgin O, Aslan G, Altintaş E, Tezcan S, Serin MS, Emekdaş G. Detection of point mutations on 23S rRNA of *Helicobacter pylori* and resistance to clarithromycin with PCR-RFLP in gastric biopsy specimens in Mersin, Turkey. Turk J Gastroenterol 2008; 19: 163-7.

16. De Francesco V, Margiotta M, Zullo A, Hassan C, Troiani L, Burattini O et al. Clarithromycin-resistant genotypes and eradication of Helicobacter pylori. Ann Intern Med. 2006; 144: 94-100.

17. Agel E, Durmaz B, Tevfik MR, Asgın R. The isolation rate and antibiotic resistant pattern of Helicobacter pylori in dyspeptic patients. Turk J Med Sci 2000; 30: 143-146.

18. Goral V, Fadile YZ, Gül K. Helikobakter pilori infeksiyonunda antibiyotik direnci. T. Klin Gastroenterohepatol 2000; 11: 87-92.

19. Graham DY. Antibiotic resistance in *Helicobacter pylori*: implications for therapy. Gastroenterology 1998; 115: 1272-1277.

20. Debets-Ossenkopp YJ, Herscheid AJ, Pot RG, Kuipers EJ, Kusters JG, Vandenbroucke-Grauls CM. Prevalence of Helicobacter pylori resistance to metronidazole, clarithromycin, amoxicillin, tetracycline and trovafloxacin in The Netherlands. J Antimicrob Chemother 1999; 43: 511-515.

21. de Boer WA. How to achieve a near 100% cure rate for *H. pylori* infection in peptic ulcer patients: a personal viewpoint. J Clin Gastroenterol 1996; 22: 313-316.

22. Demir M, Göktürk S, Oztürk NA, Serin E, Yilmaz U. Bismuth-based first-line therapy for *Helicobacter pylori* eradication in type 2 diabetes mellitus patients. Digestion 2010; 82: 47-53.