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Efficacy of Sequential Metronidazole and Furazolidone with Proton Pump Inhibitors versus Standard Regimen in Treating *Helicobacter pylori* Infection: A Randomized Controlled Trial

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Abstract

Background: *Helicobacter pylori* infection is a prevalent global health issue, especially in developing countries. Due to increasing antibiotic resistance, sequential therapy regimens have been proposed as alternatives to standard treatment. This study aimed to evaluate the efficacy and safety of a 1-week sequential regimen containing furazolidone and metronidazole compared to the standard triple therapy for *H. pylori* eradication.

Methods: In this randomized, parallel-group clinical trial, 110 patients with confirmed *H. pylori* infection were randomly assigned to either the intervention group (sequential therapy) or the control group (standard regimen). The primary outcome was *H. pylori* eradication assessed by stool antigen test 4 weeks after treatment. Secondary outcomes included adverse effects. Data were analyzed using chi-square and other appropriate statistical tests.

Results: The eradication rate was significantly higher in the intervention group than in the control group (76.4% vs. 56.4%, $\chi^2 = 4.928$, $P = 0.043$). Adverse events were less frequent in the intervention group, including nausea (16.4% vs. 23.6%, $P = 0.03$), altered taste (10.9% vs. 18.2%, $P = 0.002$), loss of appetite (7.3% vs. 14.5%, $P = 0.006$), and dizziness (5.5% vs. 0%, $P = 0.048$).

Conclusion: The 1-week sequential regimen demonstrated significantly higher eradication rates and better tolerability compared to the standard triple therapy. This approach may offer a more effective and safer option for treating *H. pylori* infection.

Keywords: Helicobacter Pylori, Furazolidone, Proton Pump Inhibitor (PPI), Metronidazole, Antibiotics

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Introduction

Helicobacter pylori is a Gram-negative bacterium predominantly residing in the human gastric antrum. Exposure to contaminated drinking water or food, particularly vegetables, increases the risk of *H. pylori* infection within populations. Although acute infection can manifest with clinical symptoms such as upper abdominal cramps, nausea, vomiting, bloating, general discomfort, and even breathing difficulties, it is important to recognize that the infection is asymptomatic in most individuals (1).

The primary risk factors for *Helicobacter pylori* infection include poor health, cultural background, dietary habits, oral and dental hygiene, crowded households, consumption

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↑What is "already known" in this topic:
Standard therapies for *Helicobacter pylori* infection face reduced efficacy due to rising antibiotic resistance and side effects. Furazolidone is a potential alternative, but concerns about its safety have limited its use. Data on short-term sequential regimens remain limited.

→What this article adds:
This study shows that a sequential regimen of metronidazole followed by furazolidone is more effective and better tolerated than standard therapy. Divided dosing and shorter duration reduced adverse effects. The findings support this approach as a cost-effective and safer option for *H. pylori* eradication.

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of unsafe water or contaminated vegetables, and swimming in rivers (2).

The prevalence of *H. pylori* infection varies across different geographic regions. It is estimated that approximately two-thirds of the global population harbors this bacterium, with a prevalence of about 35.6% reported in the United States. The highest prevalence rates have been observed among Alaska Natives (74.8%) and populations in Africa (70.1%). In Asia, prevalence ranges from 79.5% in Kazakhstan to 81% in Pakistan. Overall, roughly 70% of individuals in Africa are affected by *H. pylori* infection (3). The incidence in Eastern Mediterranean countries ranges from 22% to 87.6% (4).

H. pylori infection is recognized as a major contributor to several common gastrointestinal disorders, including gastritis, duodenal ulcers, and peptic ulcer disease (1), adenocarcinoma, and gastric lymphoma (5). Therefore, managing *Helicobacter pylori* infection is of critical importance. Standard treatment regimens typically combine common antibiotics—such as metronidazole, amoxicillin, clarithromycin, tetracycline, furazolidone, ciprofloxacin, and rifampicin—with proton pump inhibitors (PPIs) and bismuth salts. A key consideration in therapy is the bacterium's ability to develop resistance to the prescribed antimicrobial agents (6).

Eradication of *Helicobacter pylori* is crucial in developing countries, where treatment decisions often rely on drug regimens that are effective, affordable, and have minimal side effects. Key factors influencing the choice of therapy include medication cost, potential adverse effects, ease of administration, drug availability, and the likelihood of antimicrobial resistance (7). No single antibiotic can effectively eradicate *H. pylori* on its own, and the shortcomings of monotherapy have led to the adoption of multi-drug regimens, with triple and quadruple therapy being the most successful approaches (8).

A triple therapy regimen, typically including a proton pump inhibitor (PPI), clarithromycin, and either amoxicillin or metronidazole, is commonly prescribed. However, recent studies from Southern Europe and the Americas have reported reduced efficacy of this regimen, potentially due to antibiotic resistance, particularly resistance to clarithromycin (5). If the first-line treatment fails, a retreatment strategy with a four-drug regimen or sequential regimen is usually used (6).

The main challenges in treating *Helicobacter pylori* include patient tolerance and bacterial resistance to antibiotics. Initially, triple therapy achieved eradication rates exceeding 50% in patients harboring resistant strains, making it the standard first-line treatment. In cases where first-line therapy fails, retreatment strategies typically involve a quadruple regimen or are guided by endoscopy, biopsy, culture, and antibiotic susceptibility testing (3).

The sequential regimen is based on the use of furazolidone (6). Some studies have shown that high-dose furazolidone (200 mg twice daily) in the eradication regimen can cause gastrointestinal side effects, leading to a decrease in its use (6). In addition to its effectiveness, furazolidone is a relatively cheap and cost-effective drug. However, use of this drug has been limited due to its intolerable side effects such as anorexia, dizziness, rash, flu-like syndrome, and fever in 5-15% of cases (5).

Besides its efficacy, furazolidone is a relatively inexpensive and cost-effective antibiotic. Regimens incorporating furazolidone have demonstrated eradication rates of up to 90% in regions with a high prevalence of *H. pylori* infection (6), but short-term treatment courses have not been successful in Iran (9).

Conversely, antibiotics administered for *H. pylori* eradication can impact the proliferation of intestinal microbiota and modify microbial diversity. Even short-term oral antibiotic therapy in humans may alter gut microbiota for up to four weeks before the original composition is restored (10). In a multicenter study, it was found that gut microbial species in patients undergoing eradication therapy changed by more than 30% over one year (11). Another study found that following eradication therapy with vonoprazan, amoxicillin, and clarithromycin, it takes approximately three months for the diversity and composition of gut microbiota to return to pre-infection levels (12). Given the high efficacy of furazolidone in *H. pylori* treatment, the typical onset of side effects during the second week of therapy, and varying responses to different antibiotics, this study aimed to compare the effectiveness and tolerability of a furazolidone-based regimen with that of a metronidazole-based regimen, in which furazolidone and metronidazole were each administered for one week.

Methods

This study is a randomized clinical trial conducted on patients with *Helicobacter pylori* who were referred to Amir-al-Momenin Hospital of the University of Medical Sciences in 2021. A minimum sample size of 99 individuals was calculated based on the study by Khaleghi et al (13). with a 95% confidence interval and 90% statistical power, and considering the response rate in patients with severe *H. pylori* infection in the sequential regimen (64.7%) and the four-drug regimen (41.2%). Considering potential attrition, an estimated sample size of 121 individuals was determined. Finally, after a full explanation of the study and completion of the written informed consent form, 110 patients were recruited based on the inclusion/exclusion criteria with convenience sampling and were randomly allocated to each treatment group. Participants were randomly allocated, with 55 individuals assigned to group A and 55 to group B.

$$n = \frac{\left(z_{1-\alpha/2} + z_{1-\beta}\right)^2 \cdot [p_1(1-p_1) + p_2(1-p_2)]}{(p_1 - p_2)^2}$$

$Z(1-\alpha/2) = 1.96$
$Z(1-\beta) = 1.28$
$P_1 = 0.64.7$
$P_2 = 0.41.2$

The inclusion criteria were informed consent, age over 18 years, and a positive test result for *H. pylori* infection. Baseline infection status was confirmed by standard diagnostic tests prior to enrollment, and no baseline stool antigen test

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was performed since all participants had confirmed *H. pylori* infection before randomization. The exclusion criteria included pregnancy during the study, non-adherence to the medication regimen, antibiotic use in the past month, and occurrence of allergy to any of the study medications.

The data collection tool in this study was a checklist of demographic information (age, gender, marital status, education, economic status, health insurance, underlying disease history, and endoscopy history).

After obtaining approval from the ethics committee and receiving permission from the University of Medical Sciences, the researcher referred to Amir-al-Momenin Hospital. The patients with *H. pylori* were selected based on laboratory results. Participants were briefed on the study objectives and procedures and provided both written and verbal informed consent prior to enrollment. Demographic information was collected from eligible patients, and they were assured that their information would remain confidential with the researcher. Participants were also informed that the drugs would have no adverse effects on them, and they could withdraw from the study at any stage. To ensure confidentiality, a code was used for each participant.

Participants were randomly assigned to either the intervention or control group. The intervention group received metronidazole 250 mg three times daily plus 20 mg rabeprazole during the first week, followed by furazolidone 100 mg four times daily with 20 mg rabeprazole in the second week. The control group was treated with the standard regimen, consisting of clarithromycin 500 mg twice daily, amoxicillin 1 g twice daily, and rabeprazole 20 mg twice daily. After four weeks of treatment, the bacterial eradication rate was evaluated in both groups using the stool antigen test.

To minimize bias and ensure accurate outcome assessment, the study was conducted as a triple-blind trial. Participants, laboratory technicians, data collectors, and data analysts were blinded to the details of group assignment. Only the treating physician was aware of the intervention and control groups. The drugs were prepared in two packages, A (standard treatment group) and B (intervention group), by a pharmacy technician under the supervision of a pharmacist. The random allocation sequence was generated using SPSS version 22 software. The random allocation table was accessible only to the pharmacist, who assigned participants to either group A (standard treatment) or group B (intervention) based on this sequence. The primary outcome of this study was the eradication rate of *Helicobacter pylori* infection, which was assessed using the stool antigen test four weeks after the completion of treatment. Secondary outcomes included treatment adherence and the frequency of treatment discontinuation. Safety outcomes were defined as the incidence and types of adverse drug reactions reported by participants during the treatment period. Data analysis was primarily conducted on the per-protocol (PP) population, which included all participants who completed the treatment according to the study protocol (n = 110). Since no participant discontinued the treatment, the intention-to-treat (ITT) population was identical to the PP group. As all participants completed the follow-up, no imputation methods (such as LOCF) were required, and the results were analyzed based on the complete dataset.

Approval for the study was obtained from the Research Council (dated 2020, No. 6266) and the Ethics Committee of Arak University of Medical Sciences. Participants provided both verbal and written informed consent after being informed about the study objectives and the confidentiality of their data. They were also made aware of their right to withdraw from the study at any time.

Data analysis was performed using SPSS version 22 software (IBM Corp., 2013. IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.). Chi-square and independent t-tests were used to examine the distribution of demographic variables, treatment-related adverse events, and treatment response in the intervention and control groups. A significance level of less than 0.05 was considered.

To ensure allocation concealment and prevent selection bias, the random allocation sequence generated by SPSS version 22 was implemented using opaque, sealed, and sequentially numbered envelopes prepared by an independent staff member. The pharmacist opened each envelope sequentially and dispensed identical medication packages without any identifying marks. Neither patients, physicians, nor outcome assessors were aware of the group assignments prior to allocation, ensuring triple blinding. After diagnosis of *H. pylori* infection, the treating physician referred participants to the pharmacy, where the pharmacist assigned them to one of the two groups according to the randomization list.

Results

Out of a total of 121 patients, 11 (9%), due to various reasons, including not meeting the inclusion criteria and non-acceptance to participating, were not eligible to participate in the study and were excluded from the study. 110 Patients were randomly allocated to either of the two treatment groups, and finally, all patients completed the study according to the protocol (Figure 1).

This randomized controlled trial evaluated the efficacy and safety of a sequential treatment regimen consisting of one week of metronidazole plus proton pump inhibitors (PPI) followed by one week of furazolidone plus PPI compared with the standard treatment in 111 patients with *Helicobacter pylori* infection (55 in the intervention group and 55 in the control group).

Baseline demographic and clinical characteristics were comparable between the two groups (Table 1). No statistically significant differences were observed in gender distribution (female: 67.3% vs. 61.8%, $P = 0.067$), marital status (single: 45.5% vs. 36.4%, $P = 0.096$), education level (literate: 30.9% vs. 34.5%, $P = 0.117$), economic status (sufficient: 54.5% vs. 63.6%, $P = 0.71$), history of underlying disease (21.8% vs. 23.6%, $P = 0.095$), or history of endoscopy (34.5% vs. 36.4%, $P = 0.111$). The only significant difference was in health insurance coverage, with 96.4% coverage in the intervention group compared to 100% in the control group ($P = 0.002$).

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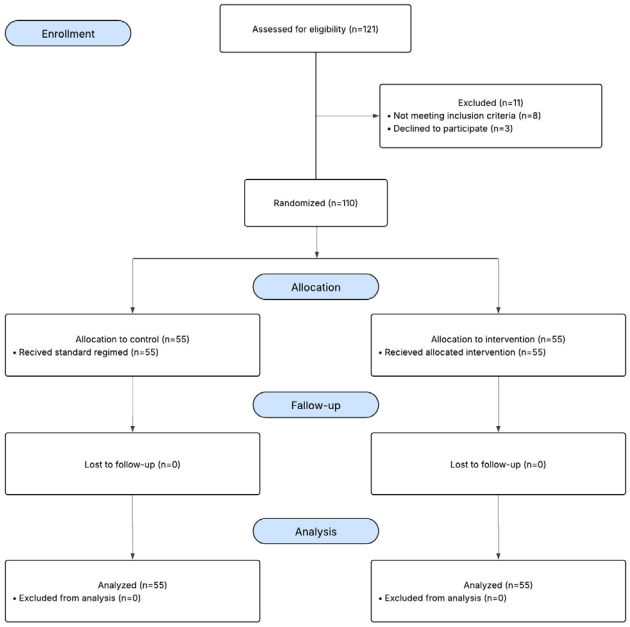

Figure 1. Consort flow diagram of patient enrollment, treatment, and follow-up

Table 1. Distribution Of Demographic Information Frequency In The Intervention And Control Groups In Patients With Helicobacter Pylori At Amir-Al-Momenin Hospital In 2021

Demographic Characteristics Of The Participants (N = 111).				
Variable		Frequency (%)		*P*-Value
		Intervention	Control	
Gender	Female	37 (67.28)	34 (61.81)	0.067
	Male	18 (32.72)	21 (38.19)	
Marital Status	Single	25 (45.45)	20 (36.37)	0.096
	Married	30 (54.55)	35 (63.63)	
Education	Literate	17 (30.90)	19 (34.54)	0.117
	Illiterate	38 (69.09)	36 (56.46)	
Health Insurance	Yes	53(96.37)	55 (100)	0.002
	No	2 (3.63)	0 (0.0)	
Economic Status	Sufficient	30 (54.55)	35 (63.63)	0.71
	Insufficient	17 (30.90)	19 (34.54)	
History of Underlying Disease	Yes	12 (21.81)	13 (23.63)	0.095
	No	43 (78.18)	42 (76.36)	
History of Endoscopy	Yes	19 (34.54)	20 (36.37)	0.111
	No	36 (65.45)	35 (63.63)	

Treatment-related adverse events were less frequent in the intervention group than in the control group (Table 2). Specifically, the incidence of nausea (16.4% vs. 23.6%, p = 0.03), taste alteration (10.9% vs. 18.2%, $P = 0.002$), loss of appetite (7.3% vs. 14.5%, $P = 0.006$), and dizziness (5.5% vs. 0%, $P = 0.048$) were significantly lower in the intervention group. Other side effects, such as headache, skin rash, dry mouth, abdominal pain, itching, vomiting, and weakness, showed no statistically significant differences between groups.

Regarding therapeutic response (Table 3), a significantly higher proportion of patients in the intervention group achieved a negative test result following treatment compared to the control group (76.4% vs. 56.4%, $\chi^2 = 4.928$, $P = 0.043$).

These results indicate that the sequential metronidazole and furazolidone regimen with PPI is both more effective and better tolerated than the standard regimen in treating *Helicobacter pylori* infection.

Discussion

According to the results of this study, the intervention group experienced a lower incidence of treatment-related adverse events, including nausea, taste disturbances, and loss of appetite, compared to the control group. Additionally, the therapeutic response was greater in the intervention group than in the control group.

Consistent with the present study, Rokkas (2021) showed that the effectiveness of combination therapy (92%) was significantly higher than standard therapy (less than 5%) (6). Moreover, in the study by Noorbakhsh (2022), the eradication rate of infection in patients treated with furazolidone was reported to be 85% (14).

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Table 2. Distribution of Side Effects Frequency in the Intervention and Control Groups

Variable		Frequency (%)		*P*-Value
		Intervention	Control	
Nausea	Yes	9(16.36)	13(23.6)	0.03
	No	46(83.64)	42(76.4)	
Headache	Yes	2(3.63)	3(5.45)	0.615
	No	53(96.37	52(94.55)	
Skin Rash	Yes	0(0.0)	2(3.63)	0.07
	No	55(100)	53(96.37	
Taste Alteration	Yes	6(10.9)	10(18.18)	0.002
	No	49(89.1)	45(81.82)	
Dry Mouth	Yes	4(7.27)	4(7.27)	0.081
	No	51(92.73)	51(92.73)	
Loss Of Appetite	Yes	4(7.27)	8(14.54)	0.006
	No	51(92.73)	47(85.46)	
Abdominal Pain	Yes	1(1.81)	2(3.63)	0.101
	No	54(98.19	53(96.37	
Itching	Yes	1(1.81)	2(3.63)	0.091
	No	54(98.19	53(96.37	
Vomiting	Yes	2(3.63)	1(1.81)	0.123
	No	53(96.37)	54(98.19	
Weakness	Yes	1(1.81)	0(0.0)	0.073
	No	54(98.19	55(100)	
Dizziness	Yes	3(5.45)	0(0.0)	0.048
	No	52(94.55)	55(100)	

Table 3. Distribution of Response To Treatment In The Intervention And Control Groups

Therapeutic Response (Negative Test Result)	Frequency (%)		Statistics	
	Intervention	Control	x2-value	*P*-value
No	13 (23.6)	24 (43.6)	4.928	0.043
Yes	42 (76.4)	31 (56.4)		

Furazolidone is a nitrofuran antibiotic that damages bacterial DNA and interferes with bacterial metabolism. Additionally, furazolidone can protect the gastric mucosa to some extent. In fact, furazolidone was widely used for the treatment of duodenal ulcers in China even before it was recommended for *Helicobacter pylori* eradication (15).

On the other hand, it seems that the reason for the higher effectiveness of combination therapy is the increased antibiotic resistance compared to clarithromycin, while bacterial resistance toward furazolidone is low. Additionally, clarithromycin is generally more expensive than furazolidone(7). Perhaps the financial capacity of patients to purchase medication and continue the treatment process is one of the reasons for the improvement in treatment response.

Bi (2022) reported a better therapeutic response to high-dose dual therapy (HDDT) (esomeprazole 40 mg and amoxicillin 1000 mg three times daily) compared to bismuth-containing quadruple therapy (esomeprazole 40 mg, bismuth potassium citrate 220 mg, and furazolidone 100 mg twice daily, combined with tetracycline 500 mg three times daily) for 14 days in patients with Helicobacter pylori infection (16). Wang (2021) also reported similar treatment response in the group receiving furazolidone and tetracycline (15). However, in the study by Matsumoto (2019), some antimicrobial drugs, including furazolidone, are not recommended for use. In the case of necessity, their dosage should be limited due to the incidence of serious side effects (7).

Based on the findings of the present study, the incidence of side effects in combination therapy was lower than that of the standard regimen. However, Bi reported a much lower incidence of side effects in patients receiving high-dose dual therapy (HDDT) compared to those receiving combination therapy with furazolidone (16). Wang also reported a higher incidence of side effects in patients receiving furazolidone compared to those receiving tetracycline. The most commonly reported side effects of furazolidone included Parageusia, Lack of appetite, Fatigue, Nausea, and Diarrhea (15).

Although Ji considers the side effects of furazolidone rare, their severity, such as sudden reduction in blood pressure, fever, headaches, and rashes, has limited the widespread use of this drug. It seems that the possible side effects are related to the inhibitory properties of furazolidone on monoamine oxidase. One of its main metabolites, amino-2-exazolidinone, can selectively inhibit the activity of monoamine oxidase, interact with tyramine metabolism, and cause gastrointestinal and nervous system disorders. Therefore, avoiding tyramine-containing foods such as aged cheeses and alcohol can prevent these side effects (17).

According to Ji (2020), most serious side effects occur with a dose of 400 mg per day in the second half of the treatment period, 10 days after starting drug therapy, and there is no high risk for side effects of low dosage (17).

In the present study, furazolidone was used in sequential therapy at a dose of 200 mg twice a day. The divided dose in the present study may be one of the reasons for the lower incidence of side effects in participants. Microbiological studies have also shown that low concentrations of furazolidone in the environment (3 mg per cc) can inhibit *H. pylori* growth without causing drug resistance. Therefore, adjusting the dose and reducing the duration of treatment seems to be a reasonable approach. However, in the present study,

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combination therapy with metronidazole was used with furazolidone. Also, the duration of treatment has been reduced to less than ten days. Therefore, it is expected that the likelihood of drug-related side effects will decrease. On the other hand, in short-term metronidazole therapy, the possibility of drug resistance to this drug also decreases (10).

In line with the present study, Matsumoto (2019) also suggests that to prevent the possibility of patient intolerance, drug allergies, and previous experiences with adverse effects, the physician should have at least two options for first-line treatment, which was done in this study by using combination therapy. The second key point is patient education about possible and expected adverse effects and the importance of compliance with antibiotic regimens (7).

One limitation of this study was the relatively small sample size. Increasing the sample size in future studies may yield different statistical results. Second, our follow-up period was short. Further research on the long-term effects of sequential therapy regimens on changes in gut microbiota can report more diverse effects.

Conclusion

This study showed that a 1-week regimen of metronidazole, rabeprazole, followed by a 2nd week course of furazolidone 400 mg/day is more effective and safer for eradicating *Helicobacter pylori* compared to the standard regimen. Additionally, the incidence of adverse effects such as nausea, taste alteration, and loss of appetite was higher in the standard treatment than in the sequential treatment. Therefore, it is recommended that healthcare providers, including physicians and nurses, provide necessary education on drug-related adverse effects.

Authors' Contributions

MT: Project development, manuscript writing. ME: Data collection. HA: Study supervision. KAS: Data analysis. LT, LGH-A: Manuscript writing and review. MRR: Final revisions. All authors approved the final version.

Ethical Considerations

The study was approved by the Ethics Committee of Arak University of Medical Sciences (IR.ARAKMU.REC.1399.315). All participants provided written informed consent.

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Conflict of Interests

The authors declare that they have no competing interests.

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