Sequential therapy versus standard triple therapy for *Helicobacter pylori* eradication

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Abstract

Helicobacter pylori (*H.pylori)* plays a crucial role in the pathogenesis of chronic gastritis, peptic ulcer disease (gastric ulcer, duodenal ulcer), gastric-mucosa-associated lymphoid tissue lymphoma (MALT) and gastric adenocarcinoma. The objective of this study is to determine whether sequential therapy eradicates *H.pylori* infection better than standard triple drug therapy for patients with dyspepsia. Patients were divided into three groups (A,B and C). Patients of Group (A) and Group (B) received sequential therapy while patients of Group (C) received standard triple therapy. Stool antigen test is used to ensure complete eradication. Results indicate that sequential therapy achieved higher eradication rates than triple therapy with lower side effects.

Key words*:* *Helicobacter pylori*, eradication, sequential therapy, triple therapy

{**Citation:** Mohammed Ahmed Abou Saif, Amira Shaban Ahmed, Nehal Abdelrahman Abdelfattah Mohammed. Sequential therapy versus standard triple therapy for *Helicobacter pylori* eradication. American Journal of Research Communication, 2015, 3(3): 132-146} www.usa-journals.com, ISSN: 2325-4076.

1-Introduction

Helicobacter pylori (HP) is a small, gram-negative spirochete inhabiting the mucous layer overlying the gastric epithelial cells in humans (De Vries AC,2010). It is the most common chronic human bacterial infection and the most common cause of gastritis with incidence rates as high as 50

% worldwide According to the World Health Organization, HP is classified as type 1 carcinogen and is the primary cause of peptic ulcer disease, gastric carcinoma and mucosa- associated lymphoid tissue lymphoma (MALT)(Malfertheiner P et al,2007). In addition, two extra intestinal entities, iron deficiency anemia (Muhsen K, Cohen D,2008) and immune thrombocytopenic purpura (Stasi R et al,2009) are currently considered related to this infection.

In Egypt, the prevalence of infection with *H. pylori* exceeds 50% by 5 years of age, and by adulthood, infection rates exceeding 90% (Robert W et al,2003).The first line treatment as suggested by international guidelines is a triple therapy, comprising a proton pump inhibitor, clarithromycin and amoxycillin or metronidazole (Chey WD, Wong BC,2007). After the initial high efficacy (eradication rate >90%) of triple standard regimens, the last decade have witnessed a progressive decline in cure rates. The high prevalence of antimicrobial drug resistance, especially to clarithromycin and metronidazole, is believed to be the key factor for this failure (Megraud F.,2004)

Helicobacter pylori treatment is difficult because there is no certain regimen able to cure the infection in all treated patients (A.Ford and P.Moayyedi ,2003). Many studies showed that standard 7-14 days triple therapies failed to eradicate *H.pylori* infection in up to 20 – 25% of patients (Fuccio L. et al,2007) with success rate less than 45- 60% in some countries (Gumurdulu Y,2004). In a recent study, the eradication rates by using three consecutive standard therapies in patients are 70.3%, 69.1% and 70% following first, second and third line regimens (Rokkas T et al,2009). It means that more efficient regimens are required to achieve high eradication rates.

In 2000, a new therapeutic regimen to cure *H. pylori* infection called 10-day sequential therapy is discovered with very high eradication rate (Zullo A et al,2000). The sequential therapy is simple dual therapy including a proton pump inhibitor (PPI) plus amoxicillin 1 gm (both twice daily) given for the first 5 days followed by a triple therapy including PPI, clarithromycin 500 mg and tinidazole (all twice daily) for the remaining 5 days. The efficacy of sequential therapy has been compared to that of standard 7-10 days triple therapies in several randomized trials. Results found that the sequential regimen was significantly superior to either 7-day and 10-day standard triple therapies with an overall eradication rate of 93.7%, 75.9% and 79.6% respectively (Zullo A et al,2007)

The aim of this study was to compare a 10- day sequential treatment regimen for *H. pylori* infection with standard triple therapy.

2-Patients and Methods

. A specifically designed form was used to record demographic and other variables that were included A total of sixty *H pylori* positive patients (16 males and 44 females) suffering from upper GIT symptoms, were enrolled in the study. The diagnosis of *H. pylori* was determined by a positive stool antigen test

Sample analysis

A stool specimen was collected in containers provided to patients . The test was done by randomly stipping the specimens collection applicator in at least three different sites to collect approximately 50mg feces which was added to an individual one ml extraction buffer solution then the specimen collection tube was shaken vigorously to mix the specimen and the extraction buffer . The tube was left alone for two minutes then the tip of the specimen collection tube was broken transferring two full drops of the extracted specimen to the specimen well of the test device, the mixture migrates upward on the membrane by capillary action to react with anti-H.pylori antibodies on the membrane and the results were read 10 minutes after dispensing the specimen.

The test is:

Positive: if two distinct colored red lines appear. One in the control line region (C) and the other in the test line region (T).

Negative: if one colored line in the control line region (C) and the absence of the colored line in the test line region (T).

Invalid: In case of failure of the control line (C) to appear which is either due to inadequate specimen volume added or incorrect procedure.

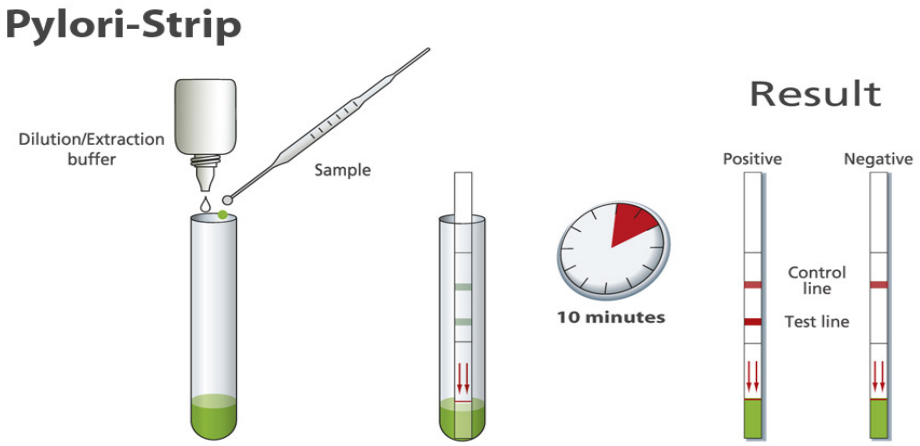

Fig.1: Stool antigen test

After receiving a complete and detailed explanation of the study, the study patients signed in an informed study. The study was approved by the Ethics Committee of Beni-Suef University teaching Hospital.

2.1: General data of patients

Inclusion criteria

The study included patients with dyspepsia or epigastric pain who were between 19-60 years of age. They had been referred for symptoms like discomfort after meal, heart burn, nausea and vomiting. Patients had not received previous *H. pylori* eradication treatments within three months prior to the referral and had not received any antisecretory drugs or antibiotics for one month prior to referral.

Exclusion criteria

Patients with serious diseases including congestive cardiac failure (CHF), cerebrovasculas diseases (CVD), decompensated diabetes, coagulation alteration and cirrhosis were excluded. Patients who had had prior gastric surgery or had allergies to penicillin were also excluded. In addition, women who were pregnant or lactating were excluded. The selected patients were subjected to full history taking, physical and clinical examination and routine laboratory assessment including complete blood count, random blood sugar, liver function tests and kidney function tests. Special test for detection of H. pylori (stool antigen test) is used.

2.2: Therapy regimens

Group A received a 10 day sequential regimen1 (omeprazole 20mg, amoxicillin 1gm) for the first 5 days followed by (omeprazole 20mg, levofloxacin 500mg, and metronidazole 500mg) for the remaining 5 days. Group B received a 10 day sequential regimen2 (omeprazole 20mg, amoxicillin 1gm) for the first 5days followed by (omeprazole 20mg, clarithromycin 500mg and metronidazole 500mg), for the remaining 5 days. Group C received a 14 day standard triple therapy of (omeprazole 20mg, amoxicillin 1gm and clarithromycin 500mg). All drugs were administered twice daily. For each therapy regimen, the proton pump inhibitor was prescribed before breakfast while all antibiotics were given after meals. Patients were asked to return at the end of antibiotic treatment to assess the compliance with the therapy and to estimate the incidence of side effects. Compliance was defined as consumption of more than 90% of drugs and was determined by pill counts.

2.3: Confirmation of eradication

Stool antigen test was repeated 4 to 8 weeks after treatment was stopped. The infection was considered to have been successfully eradicated when the results were negative.

2.4: Cost calculation

The cost of each treatment was estimated. It was as follows: 1 gm of amoxicillin, 9.5 L.E, 500 mg of clarithromycin, 30 L.E, 500 mg of levofloxacin, 7.5 L.E, 20 mg of omeprazole, 12 L.E, 500 mg of metronidazole, 6 L.E. The total cost of sequential therapy was 47 L.E (Group A), 69.5 L.E (Group B) and 152 L.E (Group c), respectively. The cost of sequential therapy was cheaper than standard triple therapy. Therefore, sequential therapy is an economic alterative to standard triple therapy.

3- Statistical Analysis

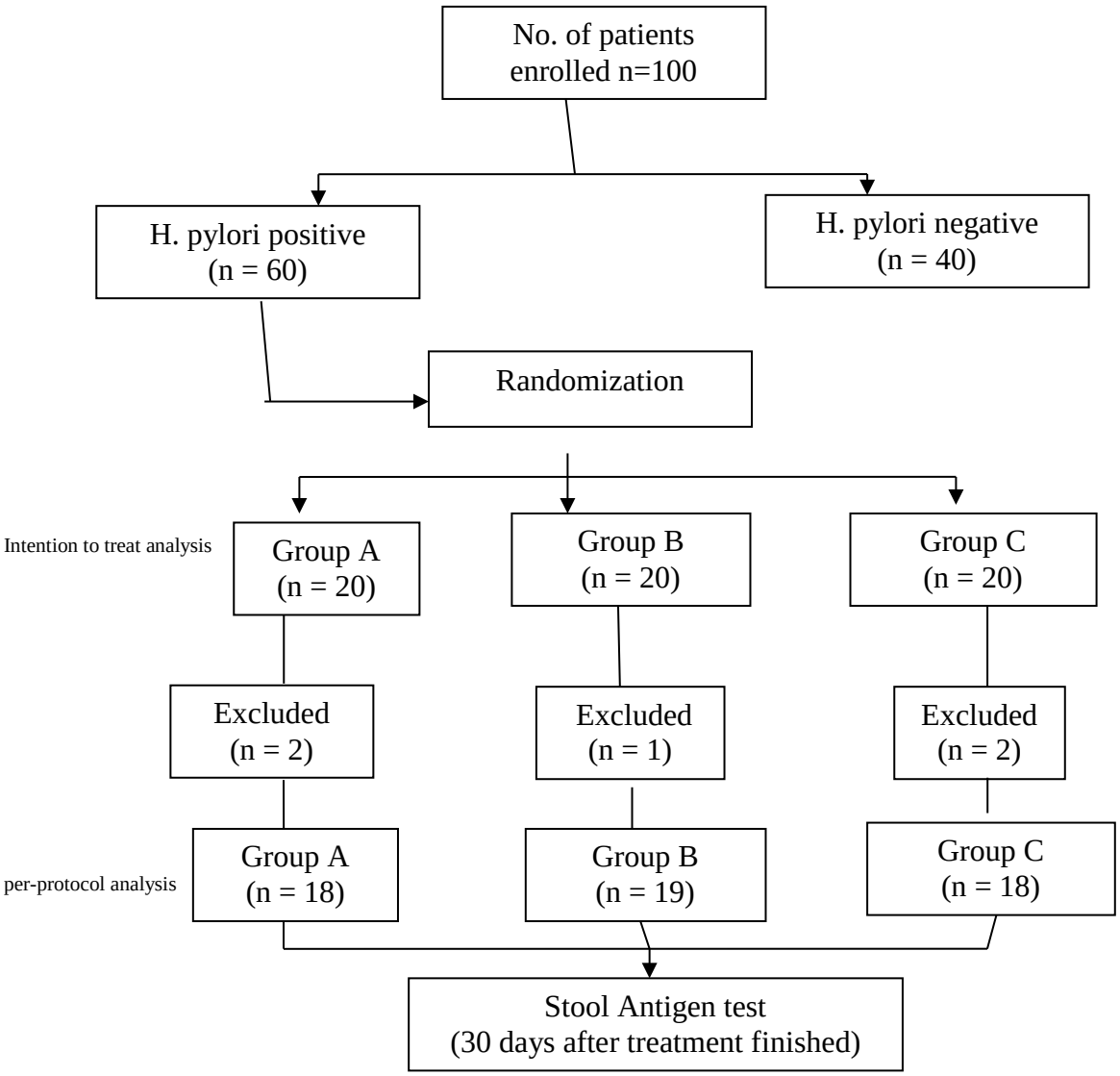

Fig.2: study design

Patients of Group (A) are excluded due to pregnancy. Patient of Group (B) is excluded due to travelling while patients of Group (C) are excluded as they did not come to complete the study.

SPSS 10 was used for statistical calculations. Comparison of qualitative variables was done by means of the chi square test or Fisher's exact test depending upon which was appropriate for the T test distribution. $P < 0.05$ was considered significant. Efficacy analyses were conducted for both intention to treat (ITT) and protocol (PP).

By hypothesizing a 95% eradication rate for sequential therapy and 80% for standard triple therapy, it was calculated that at least 18 patients per treatment arm were needed to find a statistically significant difference with a level of $p < 0.05$. *H.pylori* eradication rate and its 95% confidence interval of each scheme were calculated and analyzed by ITT (Intention to treat) and PP (per- protocol) analysis respectively. Intention to treat analysis is a comparison of treatment groups that includes all patients as originally allocated after randomization while per-protocol analysis is a comparison of treatment groups that includes only those patients who completed the treatment originally allocated.

4- Results

4.1: General Data

According to table (1) ,There were total 100 patients enrolled in the study, 60 patients accomplished actually. Details of groups devision were as follows: Group A ,20 patients , 7 males and 13 females with mean age 35.75 ± 13.37, Group B, 20 patients, 5 males and 15 females with mean age 37.25 ± 13.11, Group C, 20 patients , 4 males and 16 females with mean age 35.65 ± 13.40 . All laboratory assessments are within normal range.

Table (1): The baseline demographic characteristics of patients

Variable	Group A	Group B	Group C	P value
Age (mean ± SD)	35.75 ± 13.37	37.25 ± 13.11	35.65 ± 13.40	NS
Sex:-				
Male no.	7	5	4	NS
Female no.	13	15	16	

SBP mmHg (mean ± S.D)	127.5±11.98	128.25± 11.68	127.35±12.25	NS
DBP mmHg (mean ± S.D)	81.25±13.49	82±14.25	80.25±14.50	NS
HR /min (mean ± S.D)	81.50±13.5	82.25±13.75	80±14.25	NS
RR /min (mean ± S.D)	13.4±1.9	13.65±2.32	13.75±2.15	NS
Temp C (mean ± S.D)	36.8±2.41	37±2.53	36.6±2.65	NS
Glucose mg/dl (mean ± S.D)	79.35±8.86	79.45±8.96	79.6±8.75	NS
GPT Iu/L (mean ± S.D)	32.5±9.01	32.35±9.25	32.6±9.35	NS
GOT Iu/L (mean ± S.D)	35.6±4.32	35.5±4.45	36±4.65	NS
Serum Creatinine mg/100ml (mean ± S.D)	2.47±1.76	2.5±1.80	2.45±1.82	NS

4.2: H.pylori eradication rates

Table (2): Helicobacter pylori eradication rate with sequential and standard therapy both at intention to treat (ITT) and per- protocol analysis (PP)

Analysis	Group A	Group B	Group C
ITT (%) 95 % CI	19\20 (95%)	18\20 (90%)	16\20 (80%)
PP (%) 95 % CI	17\18 (94.4%)	18\19 (94.7%)	16\18 (88.8%)

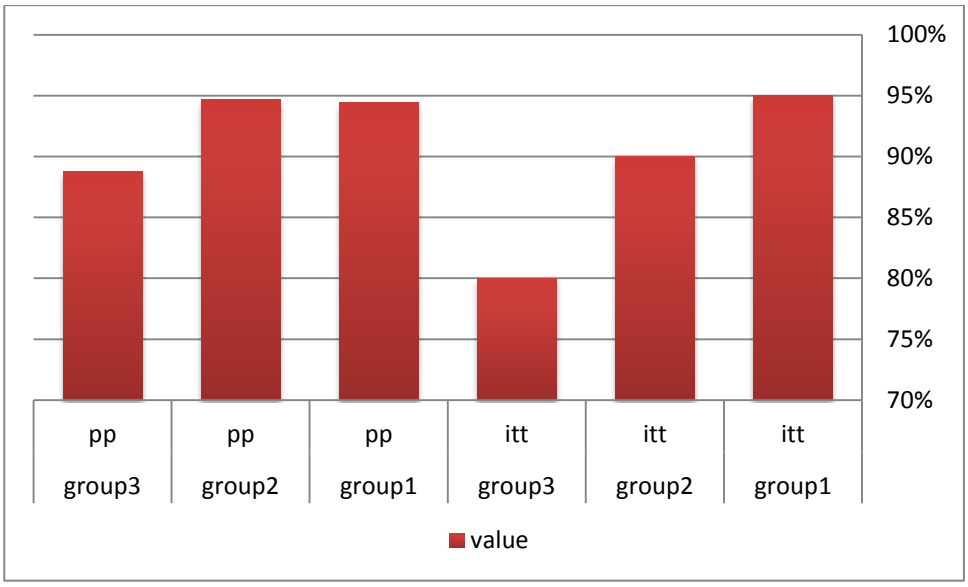

Fig.3: Eradication rates by ITT and PP

ITT analysis of group A versus group C

	Sig. (2-tailed)	95% Confidence Interval of the Difference	
		Lower	Upper
group	.0095	-10.71-	14.71
value	.0054	-7.797-	182.797

ITT analysis of group A versus group B

	Sig. (2-tailed)	95% Confidence Interval of the Difference	
		Lower	Upper
group	.074	-.48-	4.48
value	.002	69.360	107.306

ITT analysis of group B versus group C

		95% Confidence Interval of the Difference	
	Sig. (2-tailed)	Lower	Upper
group	.126	-3.85-	8.85
value	.037	21.469	148.531

According to the above tables, Group A versus Group C has significance = 0.0054 (sig < 0.05), Group A versus Group B has significance = 0.002 (sig < 0.05),Group B versus Group C has significance = 0.037 (sig < 0.05).

PP analysis of group A versus group C

		95% Confidence Interval of the Difference	
	Sig. (2-tailed)	Lower	Upper
group	.074	-.48-	4.48
value	.000	84.378	100.888

PP analysis of group B versus group C

		95% Confidence Interval of the Difference	
	Sig. (2-tailed)	Lower	Upper
group	.074	-.48-	4.48
value	.002	69.36	107.31

PP analysis of group A versus group B

	Sig. (2-tailed)	95% Confidence Interval of the Difference	
		Lower	Upper
group	.024	.48-	4.48
value	.000	69.36	107.31

According to the above tables, Group A versus Group C has significance= 0.000 (highly significant) , Group B versus Group C has significance= 0.002 ((sig < 0.05), Group A versus Group B has significance=0.000 (highly significant).

The eradication rate achieved with the sequential therapy was statistically significant compared with that obtained with standard treatment in intention- to –treat analysis (95% vs. 80%) (P = 0.0054), (90% vs. 80%) (P =0.037) and in the per-protocol analysis (95% vs. 80%) (P = 0.000), (90% vs. 80%) (P – 0.000)

4.3: Treatment Adherence

36 patients (90%) assigned to sequential treatment and 14 patients (70%) assigned to standard therapy were compliant with the medication.

4.4: Adverse effects

Both treatments were well tolerated and no patient was withdrawn from the study. The most frequent adverse effect related to the treatment was nausea. Of all the patients, group A had 10 cases, group B had 2 cases. But all the adverse reactions of each group were relatively slight. The compliance of patients was well (Table 3).

	Group A (n=18)	**Group A (n=19)**	**Group A (n=18)**
Nausea	0	2	10
Abdominal pain	1	3	2
Diarrhea	1	1	-
Total, n (%)	2 (11.1%)	6 (31.5%)	12 (66.6%)

Table (3): Adverse effects of studied groups

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	Sum of Squares	**df**	**Mean Square**	**F**	**P Sig.**
Between Groups	16.889	2	8.444	.864	.468
Within Groups	58.667	6	9.778		
Total	75.556	8			

5- Discussion

Helicobacter pylori is a common type of bacteria that usually infects people during childhood. In almost 50% of cases, the infection does not cause symptoms. However, some people with *H. pylori* infection eventually develop inflammation of stomach (gastritis) or ulcers in the stomach or small intestine (De Vries,2010). Doctors often treat stomach pain and ulcers caused by *H. pylori* with a combination of several antibiotics that are given for several days. In recent years, there has been increasing resistance to standard antibiotic treatment for *H. pylori* infection (Megraud F,2004). This means that it is harder to get rid of *H. pylori* in some patients and we need new treatment regimens.

The aim of this study is to compare the efficacy of a novel sequential antibiotic treatment with that of standard therapy for *H. pylori* eradication. All of the study participants had upper GIT symptoms and investigated for the presence of *H.pylori* by stool antigen test. Each group was given different drug regimen and follow up is made after one month.

Regimen of group A includes : Amoxicillin 1 gm twice daily + Omeprazole 20 mg twice daily for the first 5 days followed by Omeprazole 20m + Levofloxacin 500 mg+ Metronidazole 500mg twice daily for the following 5 days.

Regimen of group B includes: Amoxicillin 1 gm twice daily + Omeprazole 20 mg twice daily for 5 days followed by Omeprazole 20m + Clarithromycin 500 mg+ Metronidazole 500mg twice daily for the following 5 days.

Regimen of group C includes: Omeprazole 20 mg +Amoxicillin 1gm + Clarithromycin 500mg twice daily for 14 days.

The examination result indicated that the H. pylori eradication rate of each group was as follows: Group A (95%) , Group B (90%) and Group C(80%).

Results of group A are in agreement with a recent meta-analysis which includes four randomized controlled trials. It found that a 10-day regimen of levofloxacin-based triple therapy yielded superior eradication (95%) and was associated with fewer side effects than 7-day course of bismuth- based quadruple therapy (Saad R et al,2006).

Regarding the results of group B regimen in the present study, they showed eradication rate of 90%. This comes in agreement with several studies from Italy which reported eradication rates exceeding 90% with a novel sequential therapy consisting of PPI and amoxicyllin for 5 days followed by PPI, Clarithromycin and tinidazole for an additional 5 days. Wheather metronidazole or other imidazoles can be used in place of tinidazole has not yet been established . This regimen has achieved eradication rates superior to clarithromycin- based triple therapy and was well-tolerated in children, adults and elderly patients infected with H.pylori (Zullo A et al,2003).Group C regimen showed eradication rate of 70%, it comes in agreement with studies in United States which report intention to treat (ITT) eradication rates in the range of 70-80% (Katelaris PH et al,2002).

The reason why sequential therapy is so effective is unclear although certain hypotheses may be proposed. One possibility is that sequential treatment may exploit the advantages of both regimens. Sequential therapy for *Helicobacter pylori* refers to the idea of adding more antibiotics to the treatment regimen but giving them in a sequence rather than giving all four drugs together.

It's well known that short – term treatment (< 7 days) with proton pump inhibitor and amoxicillin can eradicate *H.pylori* in up to 50% of infected patients and reduces the bacterial load in the remaining cases (Unge P,1996). The reduction of the bacterial load may improve the response to the subsequent short course of triple therapy. Indeed, some studies have shown that a low bacterial load is associated with a higher eradication rate after triple therapy (Moshkowitz M et al,1995). Amoxicillin was chosen in the initial dual therapy phase because resistance to this antimicrobial is extremely rare and it has been found that regimens containing amoxicillin may prevent clarithromycin resistance (Murakami K et al,2002).

During the second phase, clarithromycin and metronidazole were chosen in order to exploit the efficacy of three different antibiotics. Levofloxacin can be more effective and better tolerated than proton pump inhibitor and rantidine –bismuth citrate based quadruple therapy. In the study conducted by Wong et al., levofloxacin was tested in second-line treatment in association with rabeprazole and rifabutin for 7 days. The eradication rate achieved was 91%, even if patients who

had failed to respond to more than one course of anti-H. pylori therapy were included (Wong WM et al,2003).

Uygun A and colleagues performed a study to compare the eradication success of 14- day sequential regimen with proton – pump inhibitor – based triple treatment. They found that a 14 – day sequential treatment regimen achieved a significantly higher eradication rate of *H. pylori* compared with standard PPI- based triple regimen in this selected population (A. Uygun et al, 2008).

6 - Conclusion

In conclusion, our study shows the superiority of sequential treatment for eradicating *H. pylori* infection compared with triple therapy. The sequential regimen is less expensive and more effective than triple therapy for patients with clarithromycin resistant organisms. Side effects with both regimens were similar and consisted mostly of diarrhea and abdominal discomfort. Our data suggest that sequential therapy may have a role as first line treatment for *H. pylori* infection.

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