Eradication of Helicobacter Pylori with Triple Therapy Comprising Probiotic & Proton Pump Inhibitor

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Original Research Article

ABSTRACT

Objectives: The current clinical trial compared the effects of conventional triple therapy and probiotic (Lactobacillus reuteri) plus omeprazole combination in peptic ulcer patients. The secondary objectives included estimating the effects of these regimens on safety and tolerability.
Study Design: Randomized clinical trial
Abode and Period of Study: This was a six month research study conducted at the National Medical Centre, Karachi, Pakistan in October 2020 – March 2021.
Materials & Methods: A total of 100 patients were recruited, had baseline positive stool antigen test. All the participants were separated into dual groups: conventional triple remedy (group A1) and probiotic with omeprazole combination (group B1). The study's primary endpoint was stool antigen assay and secondary included change in Hb, LFTs and renal function test.

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Results: The primary endpoints for combination therapy led to significantly greater reductions in positive stool antigen assay than triple therapy. This means that combination therapy is far better than triple therapy. The stool antigen test showed 56.5% positive and 43.5% negative in group A1 while in group B1 34.8% positive while 65.2% negative after treatment were seen with statistically significant difference (p=0.036). Insignificant findings were observed for level of Hb, LFTs and renal function test between both groups during the entire study.

Conclusion: This is the first randomized clinical trial in peptic ulcer patients of Pakistan treated with probiotic plus omeprazole combination. Combination therapy was generally well-tolerated and effective in eradicating the Helicobacter pylori after initiation of therapy.

1. INTRODUCTION

Pakistan is one of the developing countries in South Asia with occurrence of peptic ulcer disorder, which is mainly caused by *Helicobacter pylori* bacteria is found to be 85.1%. this shows that peptic ulcer is mainly associated with the presence of helicobacter pylori [1]. Hence, this emerged as the focus of Pakistani researchers' attention in identifying effective therapeutic agents to control peptic ulcer disease which can lead to serious complications like stomach bleeding, perforation, penetration to a surrounding organ and obstruction from fibrotic structuring [2]. At the time of 90s era, the conventional triple therapy was considered the benchmark in the cure of peptic ulcer disorder. The conventional triple regimen comprises of PPI, clarithromycin and amoxil. This triple therapy was recommended at the first Maastricht conference in 1996 and has been widely used for twenty years [3]. The raise in the incidence of resistance to these drugs especially to the main antibiotics, clarithromycin and metronidazole has reduced the efficacy of therapy [4]. Because of increased level of resistance and side effects of the two main antibiotics (clarithromycin and metronidazole) substitute approaches are being employed in medical practice to cure the *Helicobacter pylori* resilient strains [5].

The latest Maastricht V/Florence consensus 2016 suggested the bismuth-containing quadruple therapy in those regions where resistance to clarithromycin is very high [6]. Other remedy is Levofloxacin-based therapy; this remedy has replaced clarithromycin in standard triple regimen [7]. But due to high development of resistance to fluoroquinolone this regimen is also not used [8]. Due to these reasons, the exploration of new drug to treat *H. pylori* infection is needed.

Probiotics have affirmative impact on the abolition rates and avoidance of side effects associated with antibiotics [9]. They have confirmed to be helpful in decreasing the side effects of antibiotics and improve the patient compliance [10]. The mainly studied probiotics used for treating stomach disorders are lactic acid bacteria [11]. Lactobacillus *reuteri* have been revealed to decrease the gastric symptoms and also have antimicrobial and immunomodulatory effect [12]. Probiotic like Lactobacillus *reuteri* is a newly discovered probiotic and gain attention in recent years because of its safety. This clinical randomized trial will focus on Lactobacillus *reuteri* + proton pump inhibitor combination therapy in peptic ulcer patients.

2. MATERIALS AND METHODS

2.1 Clinical Study Design

The current randomized clinical trial was of 6 months, conducted at the National medical centre, Karachi, Pakistan. 100 patients having peptic ulcer with positive stool antigen test for *H. pylori* were recruited in the study, 92 patients successfully completed the study. They were divided into two groups based on their treatment regimens. In the group A, patients were given triple remedy (Omeprazole 20 mg two times daily + Clarithromycin 500 mg two times daily + Amoxicillin 1 gm two times daily) for two weeks, whereas the group B patients were given (Lactobacillus *reuteri* 100 mg twice daily + Omeprazole 20 mg twice daily) for two weeks.

2.2 Statistical Analysis

Sample size estimation:

Sample Size For Comparing Two Means			
Input Data			
Confidence Interval (2-sided)	95%		
Power	80%		
Ratio of sample size (Group 2/Group 1)		1	
Group 1		**Group 2**	**Difference***
Mean	5.25	4.65	0.6
Standard deviation	0.86	0.93	
Variance	0.7396	0.8649	
Sample size of Group 1		35	
Sample size of Group 2		35	
Total sample size		70	

*Difference between the means (Dore et al., 2019)

Results from OpenEpi, Version 3, open source calculator—SSMean $n = (Z_{\alpha/2}+Z_\beta)^2 \cdot 2 \cdot \sigma^2 / d^2$,

where $Z_{\alpha/2}$ is the critical value of the Normal distribution at $\alpha/2$ (e.g. for a confidence level of 95%, α is 0.05 and the critical value is 1.96), Z_β is the critical value of the Normal distribution at β (e.g. for a power of 80%, β is 0.2 and the critical value is 0.84), σ^2 is the population variance, and d is the difference you would like to detect.

The calculated sample size was 70 with 35 patients in each group. However, we have enrolled 100 patients with 50 patients in each group.

Sampling technique:

Systemic random sampling (randomized sampling technique)

Inclusion standards:

1. Male and female of age 18 to 50 years.
2. Epigastric pain 6 weeks or more duration.
3. *H. pylori* stool antigen assay test positive.

Exclusion criteria:

- Male and female of age less than 18.
- Patient with a history of cancer.
- Patient with a history of co-morbid diseases.
- Patient who are regular user of non-steroidal anti-inflammatory drugs or bisphosphonates, oral intake of antibiotics or PPIs in the precedent 3 months.
- Negative stool antigen test for *H. pylori*.

- Pregnancy or lactation.

2.3 Patients

All the peptic ulcer patients were aged 18–50 years, had epigastric pain of 6 weeks or more duration with positive *H. pylori* stool antigen assay test. Patients were excluded if they had negative test for *H. pylori*, those who had history of cancer or other chronic diseases. Patient who are regular user of non-steroidal anti-inflammatory drugs or bisphosphonates and also pregnant or lactating females were also excluded.

2.4 Endpoints and Assessments

The sample size calculation of the peptic ulcer population was estimated using Open Epi, Version 3. The primary efficacy endpoint, i.e., stool antigen assay was estimated at different intervals i.e. at baseline and after 2 weeks. The study's key secondary endpoints were complete blood count (Hb level), LFTs and renal function test. The change in liver function test (Aspartate transaminase and alanine aminotransferase), renal function test (serum urea and serum creatinine) and hemoglobin levels were quantified at baseline and after 2 weeks.

2.5 Liver Function Test (LFT)

Storage and stability: Reagents were stored at +2 to +8°C. Mix after 1 minute add start reagent 125 µl. Mix after approx. 1minute measure the decrease in absorption every minute for 3 minutes.

2.6 Calculations

The COBAS 6000/8000 system was automatically calculated the GGT activity of each patient sample. Mix after 1 minute add start reagent 125 μl. Mix after approx. 1minute measure the decrease in absorption every minute for 3 minutes.

2.7 Renal Function Test

Sample: Serum, heparin plasma, urine.

Assess the absorbance level of sample (A_S) and standard (A_{St}) after 60 sec.

3. RESULTS

The current randomized clinical trial enrolled 100 peptic ulcer patients from Karachi, Pakistan. 92 patients completed the study, 46 patients per group. Male and female between the age group of 18 years to 50 years accomplishing the inclusion criteria were involved in the research after taking the consent.

The prime objective of this research was to find out the effectiveness of conventional triple remedy vs. Lactobacillus reuteri + proton pump inhibitors combination in peptic ulcer patients of Karachi. Followed by given respective treatment regimens, stool antigen test was done. The results revealed that group A showed 56.5% positive test results and 43.5% negative test results while group B showed 34.8% positive test results and 65.2% negative test results (Table 1).

The safety and tolerability were identified by measuring Hb level, LFTS and Renal Function Test. Statistically insignificant change in hemoglobin level was found between groups at the end of the study (2-week) by showing p-value >0.05, as represented in Table 2. Furthermore, no clinically substantial levels of LFT and RFT were observed at the baseline and after 2 weeks between both groups, as shown in Tables 3 and 4.

Table 1. Evaluation between group A and group B (N= 92) (Day 14)

STOOL ANTIGEN * Group Cross tabulation					
		Group		Total	P-value
		GROUP A Conventional triple therapy	**GROUP B** Combination therapy		
STOOL ANTIGEN Positive **TEST**	Count	26	16	42	
	% within Group	56.5%	34.8%	45.7%	**0.036**[*S]
Negative	Count	20	30	50	
	% within Group	43.5%	65.2%	54.3%	
Total	Count	46	46	92	
	% within Group	100.0%	100.0%	100.0%	

Group A: treated with conventional triple therapy (Proton pump inhibitor + Clarithromycin + Amoxicillin);
Group B: treated with combination therapy (Lactobacillus reuteri + proton pump inhibitors),
P value < 0.05: significant,
*: Statistically significant, Test applied: Chi-square test.

Table 2. Hemoglobin (Hb), between group A and group B (N= 92)

	Conventional Triple Therapy	Combination Therapy	Mean Difference	P-Value
Hemoglobin (Hb; gm/L, Mean±SDev)				
Baseline	15.00±1.321	14.94±1.399		0.999
At Week 2	15.00±1.321	14.94±1.399		0.999

Group A: treated with conventional triple therapy (Proton pump inhibitor + Clarithromycin + Amoxicillin), P value < 0.05: significant,
*: Statistically significant, NS=Not Significant at 0.05.
Test applied: Independent t-test.

Table 3. Liver function test between group A and group B (N= 92)

	Conventional Triple Therapy	Combination Therapy	Mean Difference	P-Value
Alanine aminotransferase (ALT; IU/L, Mean±SDev)				
Baseline	25.67±4.033	25.46±4.037		0.999
At Week 2	25.67±4.033	25.46±4.037		0.999
Aspartate transaminase (AST; units/L, Mean±SDev)				
Baseline	28.83±5.335	29.59±4.440		0.999
At Week 2	28.83±5.335	29.59±4.440		0.999

*Group A: treated with conventional triple therapy (Proton pump inhibitor + Clarithromycin + Amoxicillin), P value < 0.05: significant, *: Statistically significant, NS=Not Significant at 0.05. Test applied: Independent t-test.*

Table 4. Renal function test between group A and group B (N= 92)

	Conventional Triple Therapy	Combination Therapy	Mean Difference	P-Value
Serum Urea (mg/dL, Mean±SDev)				
Baseline	15.43±2.208	15.24±1.923		0.999
At Week 2	15.43±2.208	15.24±1.923		0.999
Serum Creatinine (mg/dL, Mean±SDev)				
Baseline	0.83±0.167	0.83±0.163		0.999
At Week 2	0.83±0.167	0.83±0.163		0.999

*Group A: treated with conventional triple therapy (Proton pump inhibitor + Clarithromycin + Amoxicillin), P value < 0.05: significant, *: Statistically significant, NS=Not Significant at 0.05. Test applied: Independent t-test.*

4. DISCUSSION

The peptic ulcer disease can cause significant damage to the stomach mucosa and can lead to perforation and penetration of stomach mucosa and can even cause gastric carcinoma [13]. Therefore, to prevent these complications, eradication of *Helicobacter pylori* is very important. Although many treatment regimens have been proposed but conventional triple treatment is still main therapy used worldwide. But unluckily the efficacy of this management becoming reduce with time due to resistance of antibiotics mainly clarithromycin and also due to the side effects caused by antibiotics [14]. So, because of these reasons there was a strong need to find out alternative treatment regimens. Many treatment regimens have been proposed but probiotics have found to be the most suitable ones according to previous researches held on different regions of the world. That is why this research was designed to identify the effectiveness of conventional triple therapy (Amoxicillin + Clarithromycin + Proton Pump Inhibitor) versus Probiotics Lactobacillus *reuteri* + Proton Pump Inhibitor combination therapy in Pakistani population having peptic ulcer disease. As far as our knowledge, this is the first study which address the probiotic combination in the population of Pakistan.

Our study revealed that both of these interventions effectively eradicate the H. pylori in the Pakistani peptic ulcer population. But comparatively, the proportion of achieving a greater eradication rate was significantly more in combination therapy than conventional therapy. Our findings are in line with the prospective, randomized placebo trial conducted in Iran in 176 patients which disclosed that the eradication percentage of *H. pylori* was found to be considerably greater in combination therapy [15]. Another retrospective study was conducted in South Korea from March 2013 to February 2014 which showed similar results [16]. Similar outcomes were detected by Hafeez *et al*. in his study. He observed that the combination of probiotics with the triple therapy enhanced the effectiveness up to 85% and the gastric symptoms were reduced from 76% to 15% (p=0.002) [17]. A further study of 40 RCTs were conducted in which 5792 participants were recruited and they evaluated the efficacy of probiotic when used as an adjuvant to standard therapy and found that by adding the probiotic to the therapy the elimination rate of *H. pylori* has increased up to 10% [18]. In another multicentre, prospective, double-blind, placebo-controlled trial, similar results were observed. It was noted that when probiotic was combined with triple regimen the elimination rate was significantly

increased (RR = 1.13; 95%CI: 1.10-1.16; P < 0.001) [19].

Besides the efficacy of combination, another most important challenge is to prevent the onset of comorbidity or drug-induced toxicity. Therefore, in this study, the effect of both interventions on hemoglobin level was identified. The test showed insignificant mean difference in both the groups at baseline and after 14 days and the p-value was also found to be insignificant between both the groups (0.741). A former study conducted by Hassan et al., 2019 also showed no significant change between the two groups before and after treatment [20]. similar results was also showed by [21].

Next, we had identified the safety profile of these treatment regimens by liver function test (LFT). The results revealed similar findings between groups. Identifying its effect on kidney function is vital before its use. Regarding this we had checked the serum urea & creatinine levels and found that there was no change in the serum levels of urea and serum creatinine in both group A and group B at baseline and after 14 days of treatment and it showed insignificant p value for urea (p=0.520) and also for creatinine (p=0.858).

A key strength of this study is the comparison of first-line therapy, conventional triple therapy with probiotic (Lactobacillus reuteri) plus omeprazole combination for peptic ulcer treatment. Combination therapy have shown better efficacy and also helped to restore the normal microflora in the stomach. The safety profile in both groups with respect to haemoglobin, liver functions and renal functions were monitored closely. The possible limitations of the present study are the designed time period to study the effect was short and the population size of the study was also limited.

5. CONCLUSION

Probiotic (Lactobacillus *reuteri*) is clinically effective for the treatment of peptic ulcer and it has also proven to be effective in preventing the side effects associated with antibiotics in conventional triple therapy. None of the interventions elevates the incidence of cardiovascular disorder, liver toxicity and renal impairment. Probiotic (Lactobacillus *reuteri*) therapy can be used as an alternative for the treatment of peptic ulcer and for thorough eradication of *Helicobacter pylori* without producing major side effects in the population of Pakistan.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

ETHICAL APPROVAL AND CONSENT

The clinical trial was conducted after authorization by the Ethical Research Committee (ERC) of BUMDC, Karachi, Pakistan. All the patients provided written informed consent.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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