ISSN:0975-1459
Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in
Aseel Mohammed Ameen et al /J. Pharm. Sci. & Res. Vol. 11(1), 2019, 75-83

Comparative Effectiveness of Probiotics Timing Regimen in *Helicobacter pylori*- Induced Peptic Ulcer Disease Patients

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Abstract

Background: In peptic ulcer disease (PUD), *H. pylori* eradication failure rates are mostly overlapped by antibiotic resistance and alternative compounds that increase the effectiveness of treatment and/or reduce treatment-related side effects are recommended. Probiotics containing *Lactobacillus* and *Bifidobacterium* species have been suggested to improve *H. pylori* eradication.

Objectives: To explore the optimum effective regimen of probiotic supplement to *Helicobacter pylori* (*H. pylori*) eradication triple therapy in patients with *H. pylori*- induced PUD.

Patients and Methods: A prospective randomized- controlled intervention enrolling *H. pylori*-induced PUD patients allocated into four groups. Serum pepsinogen I (PGI), pepsinogen II (PGII), serum gastrin (GT), and Interleukin 10 (IL10) level were measured at the baseline and after 8 weeks. The eradication rate were evaluated by intention to treat (ITT) and per protocol (PP) analyses.

Results: Probiotic adjuvant to standard *H. pylori* eradication triple therapy produced a significant decrease in serum pepsinogen I (sPGI) level in groups 1 and 2 patients ($P < 0.05$) after 8 weeks, and a non-significant decrease in serum pepsinogen (sPGII) and PGI/PGII ratio in group 2 patients. Significant decrease in the serum gastrin in group 1 and 3 patients ($P<0.05$), meanwhile, serum IL 10 Level was not changed in all study patients ($P > 0.05$). The pooled eradication rate revealed high percentage of *H. pylori* eradication rates on ITT basis and in the PP analyses in groups 1, 2, and 3 compared to standard *H. pylori* eradication triple therapy alone, also highly significant improvement ($P < 0.01$) in patient compliance.

Conclusions: Probiotics supplement improved gastric secretions according to the effective timing regimen, and the pooled eradication rate revealed high percentage of *H. pylori* eradication rates when probiotics supplement continued after antibiotic course.

Key-words: *H. pylori* eradication, PUD, Probiotic Supplement, Standard triple therapy

INTRODUCTION

PUD refers to a defect in the gastric or duodenal mucosal wall that extends through the entire thickness of the gastrointestinal tract mucosa and muscularis mucosa caused by *H. pylori* which is the most infectious human pathogen, affecting about 50% of the population [1, 2].

PGI is a definite marker of corpus secretion capacity, and PGII is intensely influenced by degree of gastric inflammation [3-5]. These precursors (PGI and PGII) are secreted into the gastric lumen, and thus only a minimal quantity is computable in the blood, and the ratio between sPGI and sPGII (sPGI/sPGII) is considered a better marker for corpus atrophy [6]. Studies have shown that sPGI and sPGII levels are high in the presence of *H. pylori* related to non-atrophic chronic gastritis [7].

Gastrin stimulates the parietal and pepsin cells, increases gastric mucosal blood flow, and has a trophic effect on the gastric, duodenal and colonic mucosa [8]. Its main roles include food-stimulated gastric acid secretion and trophic effects on the ECL cells [9, 10].

During an acute infection leads to a transient hypochlorhydria, which facilitates the survival of bacteria and helps in its colonization of the stomach. However, chronic infection may be associated with both, hypochlorhydria and hyperchlorhydria, depending on distribution of inflammation [11]. Eradication failure rates currently exceed 20% in several countries, and it is closely associated with antibiotic resistance caused by antibiotic overuse or misuse [12-14]. Consequently, new therapies or adjunctive treatments to standard eradication regimens are needed [15].

Probiotics are live microorganisms which when administered in adequate amounts confer a health benefit on the host as defined by The Food and Agricultural Organization (FAO) and World Health Organization (WHO) [16]. The most extensively studied probiotics for treating and/or preventing gastrointestinal diseases are lactic acid bacteria, namely *Lactobacillus* and *Bifidobacterium* species [17]. The optimal and expected effect of probiotics depends on a dose and time of use as the minimal daily therapeutic dose is described as 10^6 to 10^9 colony forming unit (CFU) [18].

Probiotics have been experimentally used as single therapy in *H. pylori* eradication protocols or as therapeutic agent used concomitantly with standard eradication therapy [16].

The mechanisms of action of probiotics against *H. pylori* infection fall into four aspects, namely, first: antimicrobial substance production [19]. Second by interfering with adhesion and subsequently inhibits adhesion of pathogens [20]. Third, mucosal barrier reduced mucus secretion is frequently observed in *H. pylori*-associated gastritis [21]. Finally, immunomodulation through inhibiting cytokine reaction triggered by *H. pylori* [22]. Probiotics can modify the immunologic response of the host by interacting with epithelial cells and modulating the secretion of anti-inflammatory cytokines which may result in reduction of gastric activity and inflammation in response to *H. pylori* infection [23]. Studies reported that probiotic treatment can regulate the balance of pro-inflammatory and anti-inflammatory cytokines resulting in a reduction of gastric inflammation [24, 25].

SUBJECTS AND METHODS

Study design

An interventional prospective randomized- controlled, open-label study designed to explore the optimum effective regimen using probiotics as adjuvant supplement in patients with *H. pylori* induced PUD. The study was conducted between September 2017 to April 2018.

Patients

A total of 130 patients diagnosed with *H. pylori* infection (70 males and 60 females) with age ranges between 17 – 70 years, the patients were treated under the supervision of specialist gastroenterologist.

Only 77 patients complete the study intervention and were assessed for disease characteristic (53 patients withdrawn from the study, 23 patients due to poor compliance with the study intervention and 30 patients refused to complete the follow up) and 40 of them were objectively assessed for study parameters.

The inclusion criteria were as follows: 1) Patients with PUD confirmed by esophagogastroduodenoscopy (OGD) and 2) Patients with positive *H. pylori* test by histological examination, urea breath test (UBT), *H. pylori* antigen rapid test device (feces),

or serum antibody test. While the exclusion criteria were as follows: 1) Patients with PUD caused by NSAIDs or aspirin, 2) Patient's use of oral antibiotics, and/or proton pump inhibitors (PPIs), and/or H_2 receptor antagonists, or bismuth compound prior to the study program 3) Patients with other comorbid and 4) Pregnancy and breastfeeding.

The eligible patients were allocated into:

Group 1: include 23 patients treated with standard *H. pylori* eradication triple therapy (clarithromycin 500 mg tablets, amoxicillin 1g capsules, esomeprazole 20mg tablets) to be given twice daily for 14 days duration plus probiotic supplement to be given one capsule twice daily for 14 days", which represents 'same', i.e. probiotic administration simultaneously with the standard eradication regimen for a total period of 14 days,

Group 2: include 19 patients treated with probiotic supplement monotherapy to be given one capsule twice daily for 14 days, and then continue concurrently with the standard *H. pylori* eradication triple therapy, which represents 'before', i.e. probiotic used prior to the eradication regimen then continuing until the end of the eradication treatment for a total period of 28 days.

Group 3: include 18 patients treated with standard *H. pylori* eradication triple therapy plus probiotic supplement to be given one capsule twice daily for 14 days, followed by probiotics monotherapy for other 14 days, which represents: 'after', i.e. probiotic administered concomitantly with the eradication treatment and then continuing as a monotherapy when the eradication regimen has ended.

Group 4: include 17 patients treated with only standard *H. pylori* eradication triple therapy, which represent the control group.

Study Intervention

The probiotic supplement include 1 billion culture of (*lactobacillus acidophilus, lactobacillus salivarius, bifidobacterium bifidum* and *streptococcus thermophillus*) with the prebiotic fructooligosaccharide (FOS), designed to aid in the maintenance of probiotics activity in the intestine.

The amoxicillin and clarithromycin were administered after breakfast and dinner, esomeprazole was taken before breakfast and dinner, and meanwhile probiotic supplement was given before the breakfast and dinner. All patients continue on esomeprazole only and were followed for 8 weeks. At the end of treatment, fecal antigen test (FAT) or urea breath test (UBT) was done to confirm the eradication of *H. pylori*. Successful *H. pylori* eradication was confirmed by a negative result of UBT or FAT.

Methods

Both the *H. pylori* antigen rapid test device (feces), (CTK biotech, USA) and urea breath test were used for detection of positive *H. pylori* [26, 27]. Measurement of serum pepsinogen I (PGI) was determined using commercial enzyme-linked immunosorbent assay (ELISA) kit (Cusabio, China) [28] and serum pepsinogen II (PGII) was determined using commercial enzyme-linked immunosorbent assay (ELISA) kit (Cusabio, China) [29].Serum gastrin (GT) was determined using ELISA kit (Mybiosource, USA) [30]. Measurement of serum Interleukin 10 (IL10) was done using ELISA kit (R&D system, USA) [31]. All measurements in all patient groups was performed at the baseline and after 8 weeks treatment.

Ethical Consideration

This study was approved by the scientific and ethical committee and college of pharmacy – Mustansiriyah University. The agreement of Al-Dawly private hospital was achieved. Patient written consent was taken after full explanation of the aim of the study and ensure the reliability of the collected information.

Statistical analysis

Statistical analysis was performed using (SPSS Inc., Chicago, IL, USA, version 21). Shapiro-wilk test was done to assess normality distribution of variables. Fisher exact probability test (FEPT) was used to test the association between two categorical variables when the expected cell count is less than 5 and Pearson chi square test was used to test the association between two categorical variables when the expected cell count is more than 5. One Way Analysis Of Variance (ANOVA) to determine and find difference between K independent samples. Wilcoxon sign rank test to compare between pre and post treatment variables in same group and kruskal- wallis test to compare pre or post treatment variables between the study groups patients. The geometric mean was used to calculate the percentage of change. Analysis of *H. pylori* eradication efficacy was performed on an "intention-to-treat" basis (included all eligible patients enrolled into the study) and on a "per-protocol" basis (excluded patients with poor compliance of therapy and patients with unavailable data after therapy or patients loss due to adverse effects. Level of significance as: $P > 0.05$ not significant, $P < 0.05$ significant, $P < 0.01$ highly significant.

RESULTS

Demographic Data and Disease Characteristics of Patients with *H. pylori*-Induced PUD

The gender distribution of the study groups (female: male) ratio was as follows: Group 1 (30.43% vs 69.57%), group 2 (68.42% vs 31.58%), group 3 (38.89% vs 61.11%), and group 4 (35.29% vs 64.71%) respectively. The largest number of patients (34.7%) in group 1 were aged (31-40) years, in group 2 (26.32%) of patients were aged range (21-40) years, in group 3 (44.44%) of patients were aged (21-30) years, and in group 4 (47.04%) of patients were aged (21-30) years.

There was no statistical significant difference in the mean values of gender, BMI, age, residence, smoking habit, family history, surgical history and duration of symptoms between the study group's patients ($P > 0.05$) (Table 1).

Effect of *H. pylori* Eradication Triple Therapy Alone and in Combination with Probiotics on Serum Pepsinogen I (sPGI), Serum Pepsinogen II (sPGII), and PGI/PGII ratio

In the pretreatment baseline, there was no significant difference in the level of sPGI level among the 4 study groups ($P > 0.05$). After 8 week post-treatment with the intervention, there was statistically significant decrease in sPGI level in study groups 1 and 2 patients ($P < 0.05$) and the highest percentage of change was noticed in the probiotics-treated group 2 compared to others ($P > 0.05$), meanwhile, sPGI level in study groups 3 and 4 patients showed marked but still no significant decrease compared to pretreatment level ($P > 0.05$). There was no significant difference between study groups after 8 weeks of the treatment ($P > 0.05$).

The changes in sPGII level showed that the difference at the baseline level of sPGII level and after 8 weeks of the treatment with study intervention was statistically non-significant between the study groups ($P > 0.05$). Nevertheless, after 8-week post-treatment, there was a decrease in the level of sPGII level in study groups compared to pretreatment level, though non- significant ($P > 0.05$). The higher percentage of change was noticed in the probiotic-treated groups compared to controls on standard triple therapy ($P > 0.05$).

The changes in PGI/PGII ratio was a statistically non-significant difference at the baseline level between the study groups ($P > 0.05$). After 8 weeks from the intervention starting point, there was a statistically non-significant difference in the level of ratio PGI/PGII in study groups compared to pretreatment level ($P > 0.05$). Again, there was no significant difference between study groups after 8 weeks from the intervention starting point ($P > 0.05$) (Table 2).

Table (1): Demographic data and disease characteristics of patients with *H. pylori* induced PUD

Variable	Study groups				*P* value
	Group 1 (n=23)	Group 2 (n=19)	Group 3 (n=18)	Group 4 (n=17)	
	n %	n %	n %	n %	
Gender [a]:					
Female	7 (30.43)	13 (68.42)	7 (38.89)	6 (35.29)	
Male	16 (69.57)	6 (31.58)	11 (61.11)	11 (64.71)	
Total	23 (100)	19 (100)	18 (100)	17 (100)	0.07^{NS}
Age [b]:					
21-30	4 (17.39)	5 (26.32)	8 (44.44)	8 (47.06)	
31-40	8 (34.78)	5 (26.32)	6 (33.33)	3 (17.65)	
41-50	4 (17.39)	3 (15.79)	0 (0.00)	4 (23.53)	
51-60	5 (21.74)	2 (10.53)	1 (5.56)	1 (5.88)	
61-70	2 (8.70)	4 (21.05)	3 (16.67)	1 (5.88)	0.29^{NS}
BMI [c] kg/m^2	27.72± 4.74	28.49± 5.51	24.46±4.32	26.40±4.35	0.06^{NS}
Residence [b] :					
Rural	1 (4.35)	3 (15.79)	1 (5.56)	3 (17.65)	
Urban	22 (95.65)	16 (84.21)	17 (94.44)	14 (82.35)	0.48^{NS}
Smoking Status [b] :					
Positive	4 (17.39)	2 (10.53)	5 (27.78)	3 (17.65)	
Negative	19 (82.61)	17 (89.47)	13 (72.22)	14 (82.35)	0.63^{NS}
Family history [b] :					
Positive	5 (21.74)	5 (26.32)	5 (27.78)	4 (23.53)	
Negative	18 (78.26)	14 (73.68)	13 (72.22)	13 (76.47)	0.98^{NS}
Surgical history [b] :					
Positive	7 (30.43)	4 (21.05)	2 (11.11)	2 (11.76)	
Negative	16 (69.57)	15 (78.95)	16 (88.89)	15 (88.24)	0.42^{NS}
Symptoms duration [b] :					
< 1	12 (52.17)	12 (63.16)	7 (38.89)	6 (35.29)	
1-5	10 (43.48)	5 (26.32)	7 (38.89)	8 (47.06)	
> 5	1 (4.35)	2 (10.53)	4 (22.22)	3 (17.65)	0.44^{NS}

Data presented as Mean ±SD, (n) is number of patients and (%) is percentage (a): Pearson chi square test, (b): FEPT, (c): One way Anova test
NS: Non significant ($P > 0.05$)

Table (2): Effect of *H. pylori* eradication triple therapy alone or in combination with probiotic on serum pepsinogen I, pepsinogen II and PGI/PGII ratio

Variables	Study groups				
s P G I (ng/ ml)	**Group 1 (n=11)**	**Group 2 (n=11)**	**Group 3 (n=10)**	**Group 4 (n=8)**	***P* value**
Pre treatment	134.19	112.93	99.97	79.56	0.76^{NS}
Post treatment	87.17	39.17	84.51	49.19	0.41^{NS}
P value	0.02^{*}	0.02^{*}	0.17^{NS}	0.26^{NS}	
Percent of change (%)	-34.64 %	- 52.73 %	-43.88 %	-35.13 %	
sPGII (ng/ml)					
Pre treatment	19.89	46.46	30.15	32.49	0.63^{NS}
Post treatment	15.72	28.94	25.76	21.75	0.88^{NS}
P value	0.328^{NS}	0.09^{NS}	0.29^{NS}	0.40^{NS}	
Percentage of change (%)	-14.24%	-28.95%	-20.79%	-1.18%	
PGI/PGII ratio					
Pre treatment	5.12	2.17	3.89	3.80	0.63^{NS}
Post treatment	4.10	2.26	2.37	3.01	0.29^{NS}
P value	0.21^{NS}	0.48^{NS}	0.28^{NS}	1.00^{NS}	
Percentage of change (%)	-23.76%	-33.57%	-29.33%	-34.59%	

Data presented as median.
Wilcoxon sign rank test to compare between pre and post treatment variables in same group and kruskal- Wallis test to compare pre or post treatment variables between the study group's patients.
NS: Not significant ($P > 0.05$), $^{(*)}$ ($P < 0.05$) is considered significant.

Table 3: Effect of *H. pylori* eradication triple therapy alone or in combination with probiotic on sGT level

Variable	Study groups				
Gastrin (sGT) (pg/ ml)	Group 1 (n=11)	Group 2 (n=11)	Group 3 (n=10)	Group 4 (n=8)	*P* value
Pre treatment	539.15	292.96	539.82	244.72	0.12^{NS}
Post treatment	345.53	354.84	306.60	455.98	0.89^{NS}
P value	0.03^{*}	0.18^{NS}	0.01^{*}	0.12^{NS}	
Percentage of change (%)	-28.72 %	-19.34%	-40.38%	30.72 %	

Data presented as median.
Wilcoxon sign rank test to compare between pre and post treatment variables in same group and kruskal- wallis test to compare pre or post treatment variables between the study groups patients.
NS: Not significant ($P > 0.05$), $^{(*)}$($P < 0.05$) is considered significant.

Table 4: Effect of *H. pylori* eradication triple therapy alone or in combination with probiotic on serum IL10

Variable	Study groups				
I L 1 0 (pg/ ml)	Group 1 (n=11)	Group 2 (n=11)	Group 3 (n=10)	Group 4 (n=8)	*P* value
Pre treatment	3.45	5.92	3.56	3.95	0.60^{NS}
Post treatment	3.56	4.56	3.84	4.39	0.48^{NS}
P value	0.42^{NS}	0.17^{NS}	0.86^{NS}	0.24^{NS}	
Percentage of change (%)	-9.42%	-11.49%	7.47%	19.45%	

Data presented as median.
Wilcoxon sign rank test to compare between pre and post treatment variables in same group and kruskal- wallis test to compare pre or post treatment variables between the study groups patients. NS: Not significant ($P > 0.05$).

Table (5): Pooled Eradication Rate of *H. Pylori* infection in peptic ulcer patients

Data presented as (n) is number of patients and (%) is percentage.

Pooled Eradication Rate	Total Patients (n)	Study Groups				
		Group 1 n (%)	Group 2 n (%)	Group 3 n (%)	Group 4 n (%)	*P* value
Intention -To -Treat (ITT)	130	18 of 38 (47.36%)	17 of 37 (45.94%)	15 of 24 (62.5%)	13 of 31 (41.93%)	0.469^{NS} a
Per Protocol (PP)	77	18 of 23 (78.26%)	17 of 19 (89.47%)	15 of 18 (83.3%)	13 of 17 (76.47%)	0.729^{NS} b
Patient Compliance	100	23 of 27 (85%)	19 of 32 (59.37%)	18 of 18 (100%)	17 of 23 (73.91%)	0.007^{**} a

Where (a) Pearson chi square test, and (b) FEPT to compare between groups. NS: non-significant ($P > 0.05$) and (**) highly significant ($P < 0.01$).

Effect of *H. pylori* Eradication Triple Therapy Alone or in Combination with Probiotics on Serum Gastrin (sGT) Level

The changes in sGT level at the baseline was statistically not-significant between the 4 study groups ($P > 0.05$). After 8 week of treatment there was statistically significant decrease in the level of sGT in group 1 and 3 patients ($P < 0.05$), and non-significant decrease in group 2 patients, inversely, patients in study group 4 showed increase in sGT level compared to pretreatment level ($P > 0.05$). Overall, no significant difference between study groups after treatment ($P > 0.05$) (Table 3).

Effect of *H. pylori* Eradication Triple Therapy Alone or in Combination with Probiotics on Serum IL 10 Level

There was no significant difference before and after treatment among all study groups ($P > 0.05$). After 8 week there was also not changed notably in all study groups patients ($P > 0.05$) compared to pretreatment level [Table 4].

Pooled Eradication Rate in Patients with *H. pylori*- Induced PUD Treated with Triple Therapy Alone or in Combination with Probiotics

The Intention-To-Treat analysis (ITT) of 130 peptic ulcer patients showed that *H. pylori* eradication was (47.36%) in group 1, (45.94%) in group 2, (62.5%) in group 3, and (41.93%) in group 4 patients. There was no significant difference in the ITT between the study groups ($P > 0.05$). The per-protocol analysis (PP) analysis yield that the *H. pylori* eradication rate was (78.26%) in group 1, (89.47%) in group 2, (83.3%) in group 3 and (76.47%) in group 4 peptic ulcer patients. There was no significant difference in the PP between the study groups ($P > 0.05$). Group 1 and group 3 show higher patients compliance to study intervention (85% and 100%) respectively compared to group 2 and 4 patients (59.37% and 73.91%) respectively, and there was highly significant difference between the study groups ($P < 0.01$) in respect to patient compliance to study medications (Table 5).

DISCUSSION

Demographic Data and Disease Characteristics of Patients with H. pylori Induced PUD

Both genders have a higher rate of infection with *H. pylori* microorganism with slight predominance towards male patients in this study and others [32, 33], or towards female gender in other studies [34-36]. Moreover, *H. pylori* infection mostly presents within (21-40) years age groups in this study, and a lower prevalence rate of *H. pylori* infection was noticed in the elderly among all study groups. Two hypotheses have been proposed to explain these findings; the first, in old age *H. pylori* could be present in a small number or low activation which might not be detected, and second, the history of *H. pylori* infection may be overlapped by the development of an unfavorable gastric environment with aging [37].

Most patients in the present study were slightly overweight among the study groups. Comparable results were found in other studies where individuals with a higher BMI were more likely to be positive for *H. pylori* infection than those with a lower BMI or with an increased risk [38], and a positive relation between obesity and *H. pylori* infection were revealed [39].

Similar to the current findings, the previous study reported a high prevalence of *H. pylori infection* among patients in an urban area compared to rural one [40].

Smoking status was found to be negatively associated with *H. pylori* infection [41], and two hypotheses explained this correlation; first, after *H. pylori* infection smoking may cause spontaneous eradication by advancing atrophic gastritis, second, the elevated acid and pepsin secretion caused by smoking protects the gastric mucosa from *H. pylori* infection [42]. In this study, the nonsmokers represent the majority of PUD patients.

Positive family history of PUD was found in approximately (24%) of patients in the present study. This was consistent with the previous pilot study by Abdulridha *et al.* (2015) were approximately (26%) of *H. pylori* positive patients had a family history of peptic ulcer disease [43]. The much higher percentage was stated by Mhaskar *et al*. (2013) where (68%) of *H. pylori*-induced peptic ulcer patients had a positive family history as a risk factor in Maharashtra population [44]. With an average of (19%) peptic ulcer patients passed a positive history of gastrointestinal surgical intervention in the present study.

Majority patients in the current study presented with duration of symptoms of less than one year where the patients presented with high intensity of dyspeptic symptoms.

Effect of H. pylori Eradication Triple Therapy Alone and in Combination with Probiotics on Serum Pepsinogen I (sPGI), Serum Pepsinogen II (sPGII), and PGI/PGII ratio

As mentioned earlier, the precursors (PGI and PGII) are secreted into the gastric lumen, and thus only a minimal quantity is measurable in the blood, and the ratio between sPGI and sPGII (sPGI/sPGII) is considered a better marker for corpus atrophy [6], Also, sPGII is more sensitive to *H. pylori*-related gastritis than sPGI and is not influenced by gastritis topography. Conversely, sPGI could be stimulated by *H. pylori* infection and/or by antral G cells but also reduced by inhibiting cytokines such as interleukin-1β when the process develops in the corpus [44, 46]. Accordingly, successful eradication of *H. pylori* results in a decrease in serum PG I and PG II, however, the (sPGI/sPGII) ratio increases as a result of the PG II decrease more than that of PG I [47]**.**

The detected baseline of the present study was consistent with another study by Igarashi *et al.* (2014) where serum PGI level at baseline (73.6–253.5) ng/ml and for serum PGII level (5.15–7.25) ng/ml [48]. Also in another previous study by Di Mario *et al.* (2006) stated that serum PGI and II level at baseline was (sPGI, 130±69 μg/L ; sPGII 16±9 μg/L) in *H. pylori*-positive patients significantly higher than *H. pylori*-negative patients (sPGI 92±32 μg/L; sPGII 9±6 μg/L) [49]. The optimal cut-off value for PG I was reported in Japan and Korea studies where the cutoff value of PG I was ≤70 ng/ml and (≤3.0) for PG I/II ratio [50, 51]. On the other hand, the cut-off values were (25 µg/L) for PG I and (3.0) for PG I/ PG II ratio in European countries [52]. The difference may be due to the different methodology used to detect PG levels, but also could be due to the ethnic background of the studied population.

Large prospective 14 years baseline follow up study in the rural community of China done by Zhang *et al*. (2012) stated that serum pepsinogen status was defined as abnormal when the criteria of both serum pepsinogen I level (70 ng/ml) and a pepsinogen I/II ratio (3.0), and he revealed that the reduction in serum PG I and PGI/PGII ratio is correlated well with the severity of atrophic gastritis which is a well-known high-risk condition for developing gastric cancer [53]. In addition, it has been reported that hyperpepsinogenaemia may be considered as a subclinical marker of the genetic predisposition to duodenal ulcers suggesting that increased serum PG levels are higher risk of peptic ulcer, where the gastric ulcer rarely occurred in this population with serum concentration of PG I ≤ 100 µg/L or PG II ≤ 10 µg/L, while duodenal ulcer rarely occurred in those with serum concentration of PGI ≤ 120 µg/L or PG II ≤ 9 µg/L [54]. It is known that infection rate of *H. pylori* is extremely high in the gastric and duodenal ulcer, therefore, baseline serum PG test is a useful method for the screening and diagnosis of peptic ulcer [55].

In a histopathological sub grouping study by Kiyohira *et al.* (2003) found in *H. pylori* infection, active inflammation, and chronic inflammation, serum PGII concentrations increased and the I/II ratio decreased, meanwhile in marked atrophy or intestinal metaplasia, both serum PGI concentrations and the I/II ratio decreased, using different cut off points for the diagnosis of *H. pylori* infection (PGII ≥10 ng/ml), (I/II ratio ≤ 4.5), (PGII ≥12 ng/ml or I/II ratio ≤ 4.0) respectively [56]. Accordingly, determination of the decrease in basal pepsinogen II levels is a useful as an early non-invasive method for confirming *H. pylori* eradication, and early fall in basal gastrin values and a progressive decrease in basal pepsinogen I and II values are associated with successful eradication in patients with gastric ulcer [57]. The percentage changes in serum PG I/II ratios are useful as evaluation criteria for assessing the success of eradication therapy for *H. pylori* [48].

Following probiotic adjuvant therapy in this study, it produced a highly significant reduction in serum PG I level ($P < 0.01$) in patients receiving probiotic as adjuvant therapy (same) and patients receiving 14 days probiotic prior combining to standard *H. pylori* triple therapy (before) compared to other protocols of therapy. Meanwhile, the decrease in serum PG II was marked only in patients receiving 14 days probiotic prior combining to standard *H. pylori* triple therapy (before) compared to other protocols of therapy, though not significant ($P > 0.05$). Accordingly, the changes in the sPGI/ sPGII ratio were also decreased not significantly ($P > 0.05$) among all patients in the study groups after 8 weeks of all interventional protocols.

Plasma pepsinogen levels are expected to decrease and remain low following successful eradication. A study by Leja *et al.* in (2014) clearly demonstrated that both sPGI and sPGII levels decreased after 4 weeks of standard *H. pylori* eradication therapy where the mean sPGI level decreased (29.6%) and the mean sPGII level decrease (38.8%), meanwhile the mean PGI/PGII ratio increased from 9 up to 12.4 or by (37.8%), concomitantly with successful eradication rate [6]. Using probiotics for *H. pylori* eradication, Miki *et al.* (2007) reported that fermented milk containing *Bifidobacterium bifidum* decreases PGI and the PGI/II ratio in patients with mild mucosal atrophy, and the mechanisms by which *L. gasseri* and other probiotic bacteria influence PG levels remain unknown and require further investigation. Similar findings in the current study where the post-treatment fall in serum PG I level and in the PGI/PGII ratio in patients on the probiotic supplement (before) protocol with no change in serum PG II level [58].

The mentioned study by Igarashi *et al.* (2014) where probiotics (yogurt containing 10^9 CFU of *Lactobacillus gasseri* OLL2716 (LG21) were used concomitantly with PPIs for 3 months in patients with functional upper gastrointestinal disorders such as GERD and functional dyspepsia, the result revealed that sPGI level tended to increase following the LG21 treatment. Data suggested the possibility that LG21 induced the generation of prostaglandin E2, which then improved the symptoms via its gastro-protective effects and also increased the PGI level in the stomach [48].

Another study by Takagi *et al*. (2016) using (yogurt) as probiotic monotherapy for 3 months in *H. pylori* positive PUD patients revealed that serum PGI and PGII were significantly higher at the end of the study period compared to the baseline level, meanwhile the PGI/II ratio remained unchanged (post-treatment PGI=63.3ng/ml, PGII=22.4ng/ml, PGI/PGII=2.7) vs (baseline PGI=61ng/ml, PGII=21.4ng/ml, PGI/PGII=2.6) respectively [59].

Effect of H. pylori Eradication Triple Therapy Alone and in Combination with Probiotics on Serum Gastrin (sGT)

Abnormal gastrin production was known as hypergastrinemia which was defined as the gastrin level greater than 100–150 pg/ml [57]. High intragastric acidity inhibits the release of gastrin into circulation from antral G cells, and conversely, low acidity and high intragastric pH enhance this release [11].

A study by T. OHKUSA *et al*. (2004) reported that patients with dyspepsia had baseline serum gastrin level (131.9 ± 130.8) ng/ml in *H. pylori*-positive patients and (53.5 ± 29.2) in *H. pylori* negative patients since *H. pylori* infection of the gastric antrum up-regulates serum gastrin level [60].

The mechanisms that lead to exaggerated gastrin secretion in patients with chronic *H. pylori* infection are still unclear, hence, one possible theory is that ammonia generation by *H. pylori* urease may create an alkaline environment in the vicinity of G cells, which thus stimulates gastrin release. Another possible mechanism is that both the antral mucosal somatostatin concentration and the number of antral somatostatin-producing D cells are low in *H. pylori*-infected individuals than in uninfected controls, and since endogenous somatostatin is known to be an important inhibitor of gastrin secretion, resultant hypergastrinemia can be expected in *H. pylori*-induced PUD [61]. As the older subjects were chronic PPI-users tended to have used PPI longer and thus had a higher risk of advanced atrophy, such atrophic gastric mucosa will secrete less amount of gastric acid [48].

Previous studies have reported that elevated fasting gastrin levels decrease to baseline levels after *H. pylori* eradication therapy, BERMEJO F *et al.* (2000) stated that in gastric ulcer patients the initial mean gastrin value was (75.5 ± 39.1) pg/ml, then 1 month after successful *H. pylori* eradication treatment the level decreased to (49.2 ± 21) pg/ml [57]. Similarly, ANNIBALE B. *et al*. (2000) reported that patients with atrophic body gastritis and *H. pylori* infection, mean gastrin levels compared to after 6 months were (444.1 ± 110.7) pg/mL vs. (85.3.±28) pg/mL respectively [62].

In the current study a high baseline level of sGT was measured in all *H. pylori* positive PUD patients, thereafter treatment protocols, there was significant reduction in the level of sGT in patients receiving probiotic as adjuvant therapy (same) and patients continuing for 14 days probiotic after combining to standard *H. pylori* eradication triple therapy (after) compared to other protocols of therapy ($P < 0.01$).

The co-administration of probiotics with standard *H. pylori* eradication triple therapy and continuing for 14 days thereafter in the current study could highlight the long-lasting effect of probiotics compared with the relatively short effect of antibiotics on the gut barriers and immunity [63, 64]. Once the antibiotics were stopped, *H. pylori*-associated inflammation returns to pretreatment levels. Meanwhile, improvements after stopping probiotics were noticed months later [65, 66]. This long-term administration of probiotic induces a balance between inflammatory and proinflammatory cytokines, increasing expression of IL 10 and decreasing expression of tumor necrosis factor (TNF-α) [67-71]. Moreover, the probiotics could secrete antibacterial substances in addition to lactic acid which maintains high intragastric acidity inhibits the release of gastrin into circulation from antral G cells [72].

Myllyluoma *et al*. (2007) reported that therapeutic intervention using probiotics combination decreased the sGT-17 level in *H. pylori* infected patients, and several probiotic strains have shown to decrease IL-8 and TNF-α, and hence, interfere with a gastrin-17 release during *H. pylori*-induced inflammation [7].

Previous experimental study done by Uchida *et al.* (2004) mentioned that yogurt containing LG21 significantly increased the prostaglandin generation in the gastric mucosa in rats, such as prostaglandin E2 and prostaglandin I2, are known to exert a gastro-protective effect by increasing the gastric mucosal blood flow or bicarbonate secretion [72]. Moreover, prostaglandin E2 is reported to stimulate chief cells to secrete PGI via the cAMP pathway [48].

Effect of H. pylori Eradication Triple Therapy Alone and in Combination with Probiotics on Serum IL 10

It has been suggested that the chronic gastric mucosal inflammation induced by *H. pylori* potentially have systemic effects based on the increase in serum pro inflammatory cytokines [73]. IL-10 inhibits the synthesis of Interferon (IFN-c), IL-1, IL-6, IL-8 and TNF-α, and also acts as a feedback mechanism in reducing these cytokines [74].

A study by Abdollahi *et al.* (2011) reported that IL-10 was a key modulating agent during host response to infection, IL-10 is like a natural suppressor in immune reactions, and there was no significant difference in IL-10 levels in mucus (antrum and body) and in serum. In addition, this cytokine in most of the patients was undetectable. This may probably be due to the low effect of (T-helper2) Th2 cell response to *H. pylori* infection [75].

Another study by Siregar *et al.* (2016) stated that the serum levels of IL-10 increased significantly in *H. pylori* positive patients, with a mean level of (1.62±1.56) pg/ml ($P < 0.05$) compared to negative *H. pylori* patients with mean level of (1.39 ±2.32) pg/ml [74].

The current study revealed that the baseline serum IL10 level was less than the detection range, and no significant change in the level of serum IL10 ($P > 0.05$) after *H. pylori* eradication triple therapy alone or in combination with probiotics.

The anti-inflammatory effectiveness of probiotics namely the Lactobacilli on the gastric mucosa through local or systemic immunomodulation, some lactic acid bacteria (LAB) can prevent the appearance of local inflammatory diseases, and the stimulation of IL-10-producing cells is strain-dependent traits, and also depends on the concentrations used and the method of administration [76].

In a previous study by Myllyluoma *et al*. (2008) to explore the effects of multispecies of probiotic combination on measurement of interleukin-8 (IL-8), IL-10, prostaglandin E2 (PGE2), and leukotriene, the proinflammatory actions of the individual components dominated the anti-inflammatory effects when the probiotic bacteria were used in combination, and the therapeutic response can be optimized if probiotic strains are characterized before they are used in combination [77].

In an *in vitro* study demonstrated that the probiotic BIFICO cocktail (composed of viable *Enterococcus faecalis*, *Bifidobacterium longum,* and *Lactobacillus acidophil*us) resulted in reduced levels of cytokines and chemokines relevant to gastritis, such as TNF-α, IL-1β, IL-10, IL-6, granulocyte colony stimulating factor (G-CSF) and macrophage inflammatory protein (MIP-2) [73].

Moreover, in a recent *in vitro* study by Sichetti *et al*. (2018) reported anti-inflammatory property of a mixture of probiotics (*l. rhamnosus, b. lactis* and *b. longum)* to prevent inflammation through inducing IL-10 and reducing pro-inflammatory cytokines production [78].

Pooled Eradication Rate of H. pylori in PUD Patients on Triple Therapy Alone and in Combination with Probiotics

The current study revealed that administration of probiotics, either (same , before, or after) timing regimen with standard *H. pylori* eradication triple therapy, high percentage of *H. pylori* eradication rates on ITT basis compared to standard *H. pylori* eradication triple therapy alone (47.36%) (45.94%) (62.5%) vs (41.93%) respectively. Also the eradication rates in PP analysis in patients receiving 14 days probiotics prior combining to standard *H. pylori* triple therapy (before) was (89.47%), and in patients continuing for 14 days probiotic after combining to standard *H. pylori* eradication triple therapy (after) was (83.30%), both percentages were found to be higher than patients receiving probiotics concomitantly with the standard *H. pylori* triple therapy (same) (78.26%). All probiotic timing regimen showed a higher percentage of eradication rate compared to the control group patients, though not significant. Nevertheless, the pooled data related to the patient compliance revealed (100%**)** in patients continuing for 14 days probiotic after combining to standard *H. pylori* eradication triple therapy (after) and in patients receiving probiotics concomitantly with the standard *H. pylori* triple therapy (same) (85%), the difference was highly significant ($P < 0.01$) compared to other groups. In a very recent systematic review with pooled-data analysis, Losurdo G *et al.* (January 2018) found that probiotic may eradicate *H. pylori* in (14%) of cases as a monotherapy. *Lactobacilli, Saccharomyces boulardii,* and multistrain combinations eradicated the bacterium with a rate of (16%, 12%, and 14%), respectively, and treatment with probiotics was significantly more effective than placebo (OR = 7.91) [79].

The findings of the present study was consistent with that of Du YQ *et al.* in (2012) who reported the use of probiotic contained *acidophilus*, *Streptococcus faecalis* (*S. faecalis*) and *Bacillus subtilis* (*B. subtilis*) either (before or after) the standard *H. pylori* triple therapy enhanced eradication rates, where PP analysis of (81.6%) was significantly higher in the patients received pre-treatment for two weeks of with probiotics, prior to *H. pylori* eradication triple therapy for 7 days (before), also PP analysis of (82.4%) in patients received (after) timing regimen, both compared to (61.5%) in patients receiving the standard *H. pylori* eradication triple therapy. The ITT analysis was significantly higher (79.5%) and (79.2%) vs (60.8%) respectively [80].

Previous studies suggested that probiotic supplementation may increase eradication rates when provided prior or subsequent (before or after) to any standard *H. pylori* eradication regimens, but concurrent supplementation did not significantly improve the eradication rate. The reason behind that antibiotics discontinuation may restrain the growth of the probiotics, resulting in a decrease in the anti-*H. Pylori* substances produced by the probiotics [81, 82]. Previous study by G. Scaccianoce *et al.* (2008) evaluated the high concentration of *Lactobacillus reuteri* (*L. reuteri*) probiotic in addition to standard *H. pylori* eradication triple therapy for 14-days, no therapy regimen achieved (>80%) eradication rate at both intention-to-treat (ITT) and per protocol (PP) analysis [83].

The meta-analysis by LV *et al.* (2015) stated that the pooled eradication rate of the probiotic supplement was (80.3%) in ITT analysis, and (83.8%) in PP analysis, with a total eradication rate in the probiotic group was higher than that in the control group (80.3 vs. 72.2%) with a statistically significant difference ($P<0.001$), and the duration of probiotic supplementation for >2 weeks could significantly improve the eradication rate for *H. pylori* infection, meanwhile a duration of ≤ 2 weeks could not. This indicated that the long-term administration of probiotics could be beneficial during *H. pylori* treatment.

A total of 21 study based on the timing of probiotic supplementation ('before', 'same' and 'after'), eradication regimens, duration of probiotic supplementation, species of probiotics, age of patients (adults and children) and Jadad scores were available, hence, none of them compared the effectiveness of the three protocols regarding the timing of probiotic supplementation in one study [84].

Both Cindoruk *et al*. (2007) and Medeiros *et al*. (2011) concluded that simultaneous probiotics administration with the *H. pylori* eradication regimens resulted in higher eradication rate compared to controls **(**83.9% vs 71%) and (80.6% vs 59.7%) respectively [85, 86].

On the other hand, Kim *et al*. (2008) and Deguchi *et al*. (2012) stated that using probiotics prior to the *H. pylori* eradication regimen and continuing until the end of the eradication treatment produce an increase the eradication significantly (82.6% vs 69.3%) in one study and non-significant increase (79.2% vs 72%) in the other study [87, 88].

Moreover, Sheu *et al*. (2002) and Song *et al*. (2010) studied the administration of probiotics concomitantly with the eradication treatment and continuing subsequent to the end of the eradication treatment, the eradication rate of ITT analysis were significantly higher in the patients receiving probiotics than the patients on *H. pylori* eradication treatment only (91.3% vs 78.3%) and (80% vs 71.6%) respectively [89, 90]. Controversially, Armuzzi *et al*. (2001) and Myllyuioma *et al*. (2005) stated that the later probiotics protocol (beginning with the *H. pylori* eradication treatment and continuing subsequent to the end of the eradication treatment) did not produce significantly higher eradication rate of ITT analysis (83.3% vs 80%) and (91% vs. 79%) respectively [91, 92].

CONCLUSION

This study revealed that probiotics supplement improved gastric secretions and accordingly symptom relief depending on the affective timing regimen particularly when given before and continue concomitantly with standard *H. pylori* eradication therapy, meanwhile the pooled eradication rate revealed high percentage of *H. pylori* eradication rates when probiotics supplement continued after antibiotic course.

Acknowledgment

The authors would like to thank all participants for providing the practice platform of this study.

Conflicting Interest

The authors report no conflicts of interest in this work.

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