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A Prospective Randomized Interventional Study to Establish the Role of Bovine Lactoferrin in Helicobacter Pylori Infection

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Abstract

Helicobacter pylori (H. pylori) are gram-negative spiral bacterium that surrounds itself in the epithelial surface of the stomach. It is found to be the most common bacterial infection affecting the population worldwide approximately 50%. The elimination of *H. pylori* is not only an important component in the healing of peptic ulcers, but also to decreases their recurrence and reduces the rate of gastric carcinoma. Eradication of *H. pylori* is currently recommended in numerous guidelines for the treatment of all these associated diseases. In the last two decades, attention has focused on the potential role of bovine lactoferrin (bLf) in the therapy of various gut disorders. bLf, a multifunctional iron-binding glycoprotein that is found in the milk, mucosal secretions (e.g., saliva, tears, bile), pancreatic and seminal fluids, and specific granules of the polymorphonuclear leukocytes in humans. Proton-pumpbased triple therapy (PpTT) is the most commonly accepted *H. pylori* treatment, which consists of a twice-daily proton pump inhibitor (PPI) such as esomeprazole or pantoprazole or omeprazole; clarithromycin or amoxicillin with tinidazole for 7-14 days. The aim of our study was to evaluate the efficacy of Bovine lactoferrin, administered orally along with triple therapy on *H. pylori* colonization in humans by a prospective randomized controlled study. 75 Patients with *H. pylori* positive were recruited and treated as per the protocol for 2 weeks. The subjects were randomized into 2 groups based on inclusion and exclusion criteria, interventional group received bovine lactoferrin at a dose of 100mg twice daily along with triple therapy for 14 days and the control group received, triple therapy for the same period of 14 days. The reduction in symptoms were analyzed by using a set of questionnaire and scored by using Global Overall Symptom Scale (GOS) both prior and post treatment. The eradication of *H. pylori* was confirmed by (13c) Urea breath test (UBT) that has shown the eradication rate of 76.2% in interventional group and 61.1% in control group. This study results had indicated that the group received bovine lactoferrin had shown significant improvement in the *H. pylori* eradication. This result can be used to conclude that the role of bovine lactoferrin in the reduction of symptoms and eradication of *H. pylori* infection by focusing on the relatively high cure rate, good compliance to the treatment and higher quality of life.

Keywords: H. pylori; bovine lactoferrin; Urea breath test; triple therapy

Introduction:

Helicobacter pylori is a gram-negative spiral bacterium that localize itself in the epithelial layer of the stomach. It is one of the most common bacterial infections in the world affecting approximately 50 % of the population[1].The prevalence was found to be increasing in developing countries and can rise to 80-90% where some with a serious disease, while others are asymptomatic[2]. The major route of transmission for Helicobacter pylori infection are fecal-oral,oral-oral, contaminated food and water where ones acquired, the infection is typically lifelong when it is not treated[3].The eradication of H.pylori plays a vital role in the healing of the peptic ulcer where it reduces the rate and recurrence of gastric carcinoma. The invasive diagnosis of H.pylori is by biopsy specimens obtained during endoscopic examination for a rapid urease test. Non-invasive tests included antibody deduction in serum specific to H.pylori, 14C(radioactive), and 13C(non-radioactive) urea breath tests. H.pylori eradication therapy is currently recommended in numerous guidelines for the treatment of all these associated diseases. The most common eradicating treatments include triple and quadruple therapy in which triple therapy consists of two antibiotics and one proton pump inhibitor whereas in quadruple therapy bismuth subsalicyalte with two antibiotics and one proton pump inhibitor are used. Proton pump-based triple therapy is the most commonly used eradicating treatment where drugs like clarithromycin and amoxicillin/metronidazole are used as an antibiotic with pantoprazole, esomeprazole omeprazole, rabeprazole, and lansoprazole as a proton pump inhibitor for 7-14 days[3]. The foremost reason for the failure of eradicating therapy is due to poor patient compliance and bacterial resistance. The frequency of mild but unpleasant side effects such as metallic taste, diarrhea and nausea are reported highly and therefore makes poor compliance of the prescribed antibiotic course which ultimately leads to bacterial resistance. This circumstance has forced the researcher to look into alternative solutions with more patient compliance for eradicating H.pylori[4].In the last two decades, recognition has been made towards the role of bLf in various gut disorders. bLf is found in the milk, mucosal secretions (e.g., saliva, tears, bile), pancreatic and seminal fluids, and specific granules of the polymorphonuclear leukocytes in humans which is a multifunctional iron-binding glycoprotein. bLf has an antibacterial property that helps the host to defense against a wide range of bacteria[3]. Due to its high iron-binding affinity, it prevents iron uptake by the bacterium, by this it removes an important nutrient required for pathogen growth and acts as a bacteriostatic and bactericidal agent. bLf also provides bactericidal effects by binding to the outer membrane of gram-negative bacteria and triggering the release of lipopolysaccharides which causes osmotic damage. An explicit increase in the eradication rate and a reduction in side effects were observed when bLf was added to the standard 1-week triple therapy against H. pylori[4].

This study aimed to establish the efficacy of bovine lactoferrin in patients with Helicobacter Pylori infection who are treated with triple therapy.

Patients,Material and Methods:

This study was a prospective, open-label, single-center, randomised controlled study. The study here enrolled 75 consecutive patients who were newly diagnosed to be H.pylori positive from gastroenterology unit of multispeciality tertiary care private corporate hospital during the study period i.e July-2022 to October 2022. A Statistically calculated 90 patients were the sample size and the it is

calculated, with a 95% confidence level, with the marginal error of 0.03 and success rate /proportion with 0.02.

All the patients who referred to gastroenetrology department were given a thorough physical examination, and those who had severe gastrointestinal symptoms have undergone a upper endoscopy, during which biopsy samples are taken from antrum and tested for H. pylori by rapid urease test.The gastric biopsy test is based on the activity of the H.pylori urease enzyme, which splits the urea test reagent to form ammonia. Ammonia increases the pH, which is detected by the indicator phenol red. These test gives result in 1hour to 24 hours depending upon the format of the test. The RUT dry Test (Gastro cure system, Kolkata, India) was used in our study to confirm the H.pylori infection.
A total of 75 consecutive patients were found to have H.pylori infection based on upper endoscopy with rapid urease test.

Inclusion Criteria: Both inpatient and outpatient of the study site with age of ≥18 years, either of gender and diagnosed with Helicobacter Pylori infection.

Exclusion Criteria: Patient below age of 18 years, previously diagnosed with *H. pylori* and who were on treatment with *H.Pylori* kit and/or antibiotics preceding the enrollment, patients with active GI bleeding at the time enrollment, patients undergone prior eradication treatment, patients with the history of Gastrectomy, esophageal surgery, definitive acid lowering surgery, advanced liver or renal diseases and any form of malignancy, patients with proven clarithromycin or any antibiotic allergy, and pregnant/lactating women were excluded from the study.

All patients regardless of their upper gastro endoscopic and rapid urease results were categorized into two groups and followed same 14 days eradication therapy. They were randomly categorized into two groups control group (n=29) and interventional group (n=46). The participants with in interventional group is treated with standard triple therapy with the addition of bLf (b.i.d) for 14 days. bLf was taken in the form of commercially availabe tablet (Neuronomic) containg 100mg of bLf and guanosine 5-monophosphate10mg. The control group was given with HP kit(amoxycillin+tinidazole+omeprazole)/sompraz hp(clarithromycin+esomeprazole+amooxycillin) . participants are advised and instructed to take the proton pump inhibitors 30 mins before the food,thye antibiotics and bLf were advised and instructed to take 30 mins after the food.

The study was performed in accordance with good clinical practice and the Declaration of Helsinki guidelines, and all patients gave their informed written consent before participating in the study.Each was carefully instructed regarding the drug protocol and the importance of following it without deviation.In addition, general explanations concerning the rationale and importance of an accurate intake of medication, a detailed description of the treatment, and a daily table of drug consumption, were given to the patients

Wilcoxon signed rank test was carried out to determine the efficacy of the two treatment groups . Results were considered statistically significant for *P* values less than 0.05.Baseline characteristics of subjects between groups were compared by using chi-square test.

Questionnaire:

A survey using a questinnaire was conducted among the study participants to evaluate the percentage of symptomatic relief before and after the completion of treatment. The questionnaire was assessed using Global Overall symptom rating scale with scoring of 1-7 based on their pain sensation felt by the patients. In which **1** indicates **No problem** and **7** indicates **Very severe problem**. The participants has to choose the score based on their satisfaction prior to the treatment and 14 days post the treatment. The higher the pain score the higher intensive care is essential for the patients. The symptomatic relief from the treatment can be seen through the evaluation of symptoms. The questionnaire consists of various symptoms associated with the *H. pylori* which includes abdominal discomfort, epigastric pain, acid reflux, nausea, belching, early satiety, heartburn, bloating, regurgitation, vomiting, anorexia and indigestion.

At the end of 14 days of treatment, patients of both groups were subjected to UBT to confirm the eradication of *H. pylori*. A negative urea breath test indicates h.pylori eradication.

Results:

Interventional group consisted of 29 patients (men 24 and women 4, mean age **45 ± 15.369**) and control group of 46 patients (26 men and 40 women, mean age **44.22 ± 15.789**).No significant differences in the demographical characteristics (mean age $P = 0.832$, but significant difference was found between male to female ration in both groups (M:F ratio $p = 0.007$. There were no significant difference between comorbidites in both group ($p = 0.626$) and upper endoscopy (OGD scopy report findings) results showed that small hiatus hernia were found to be significant in both groups.

14 days after completion of treatment both group underwent urea breath test.The urea breath test was done after the treatment to assess the *H. pylori* eradication. Of the total number of 75 participants, 39 underwent urea breath test (21 in interventional group and 18 in control group). The remaining participants were lost due to following reasons:

1. Participants who did not report after 14 days of treatment.
2. Participants who were not ready for the test and they felt very better after the treatment.
3. Participants who had lack of time and interest.
4. Participants who had fear of one additional procedure.

The urea breath test results revealed eradication rate of 76.2% in 21 participants in interventional group and 61.1% in 18 participants in control group. The urea breath test reports revealed that the eradication rate was higher in interventional group when compared to the control group. The Wilcoxon Signed Ranks test performed had revealed that the ‘p’ value was found to be 0.309, which indicates that there is no statistical significance between the data.However, benefit of adding bLf to the triple therapy is evident when there is equal number of patients enrolled in urea breath test.

The overall assessment of prior and post treatment questionnaire: The symptomatic relief questionnaire were given to the participants in two intervals (prior and post the treatment). The questionnaire were assessed in all 75 participants. The wilcoxan signed rank test was used to assess the symptom scoring given by each participants. The p value indicates the level of significance, where the **p**

value of <0.05 states that the result is significant and **p value of < 0.001 s**tates that **very significant difference is found and it is indicated through - ** .** The overall result of the symptomatic relief assessment by questionnaire were found in **table no** and the results indicates that there is overall improvements is higher in interventional group when compared to the control group.

Table 1: p value for both interventional and control group using wilcoxon signed rank test

	Intervention		Control	
	Z	Asymp. Sig. (2-tailed)	Z	Asymp. Sig. (2-tailed)
abdominal pain (Post vs. Pre)	-4.731 a	0.000**	-6.098 a	0.000**
epigastric pain (Post vs. Pre)	-4.458 a	0.000**	-5.239 a	0.000**
acid reflex (Post vs. Pre)	-3.859 a	0.000**	-4.680 a	0.000**
nausea (Post vs. Pre)	-2.719 a	0.007*	-3.419 a	0.001*
belching (Post vs. Pre)	-4.001 a	0.000**	-4.074 a	0.000**
early satiety (Post vs. Pre)	-3.352 a	0.001*	-4.192 a	0.000**
heart burn (Post vs. Pre)	-3.984 a	0.000**	-4.548 a	0.000**
bloating (Post vs. Pre)	-3.756 a	0.000**	-4.146 a	0.000**
regurgitation (Post vs. Pre)	-3.441 a	0.001*	-4.377 a	0.000**
vomiting (Post vs. Pre)	-2.827 a	0.005*	-4.375 a	0.000**
anorexia (Post vs. Pre)	-3.328 a	0.001*	-3.573 a	0.000**
indigestion (Post vs. Pre)	-3.844 a	0.000**	-4.481 a	0.000**

a. Based on positive ranks.
b. Wilcoxon Signed Ranks Test
*** - significant if p < 0.05**
**** - significant if p < 0.001**

Table 2: Eradication rate using Urea Breath Test

UBT	Interventional Group Percentage (Frequency)	Control Group Percentage (Frequency)	Wilcoxon Signed Ranks Test p value
Positive	23.8% (5)	38.9% (7)	0.309
Negative	**76.2% (16)**	**61.1% (11)**	
Total	100.0% (21)	100.0% (18)	

The above data is pictured in next graph.

Figure 1: Eradication rate using Urea Breath Test

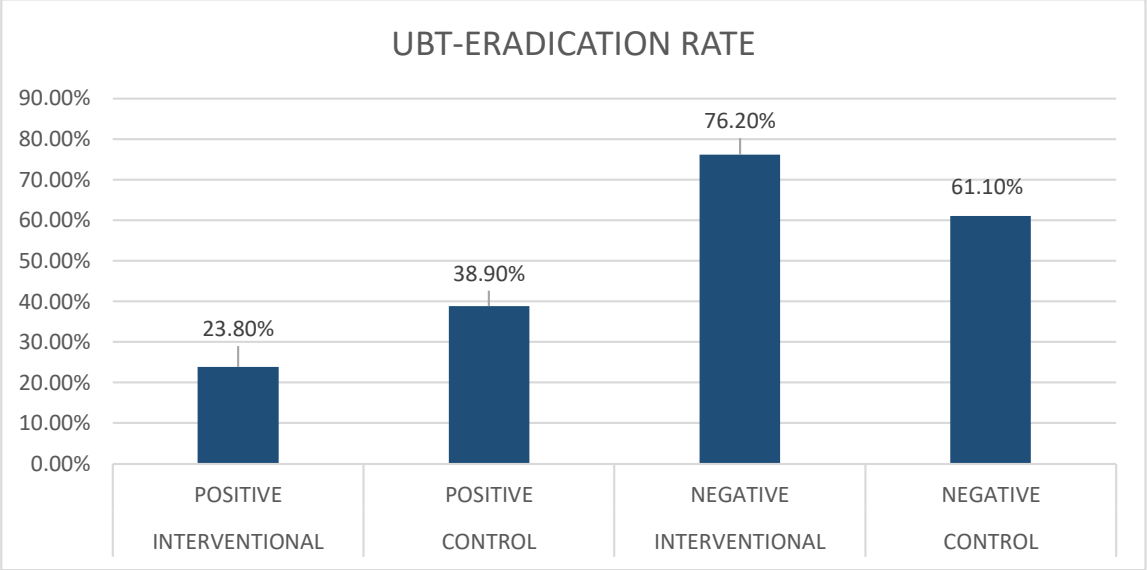


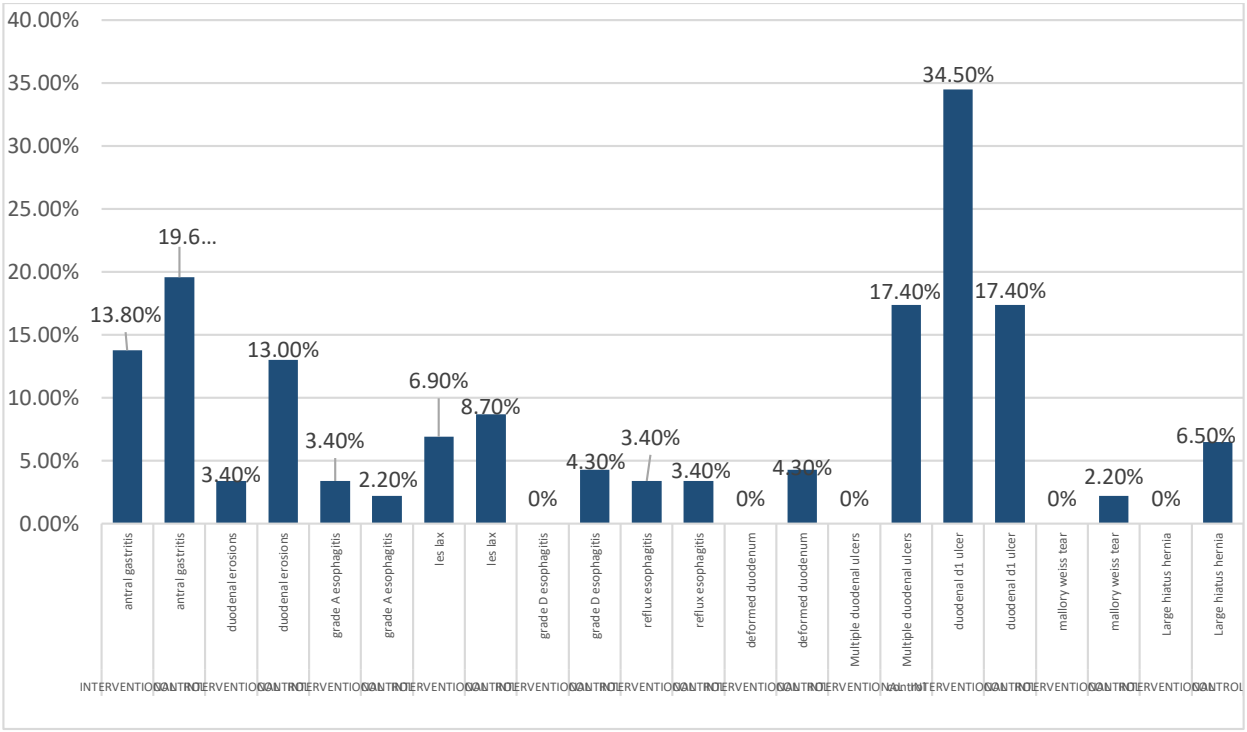
Table 3:Characteristic of ODG scopy results of both control and inverteintional group participants

CHARACTERISTIC (control)	FREQUENCY	PERCENTAGE
Small hiatas hernia	37	80.4%
Pan gastritis	5	10.9%
Antral gastritis	9	19.6%
Duodenal erosions	6	13.0%
Grade A esophagitis	1	2.2%
Les lax	4	8.7%
Grade D esophagitis	2	4.3%
Reflux esophagitis	1	3.4%
Deformed duodenum	2	4.3%
Multiple duodenal ulcers	8	17.4%
Duodenal d1 ulcer	14	30.4%
Mallory weiss tear	1	2.2%
Large hiatus hernia	3	6.5%
CHARACTERISTIC(interventional)	FREQUENCY	PERCENTAGE
Small hiatas hernia	10	34.5%
Pan gastritis	4	13.8%
Antral gastritis	4	13.8%
Duodenal erosions	1	3.4%
Grade A esophagitis	1	3.4
Les lax	2	6.9
Grade D esophagitis	-	-
Reflux esophagitis	1	3.4
Deformed duodenum	-	-

Multiple duodenal ulcers	-	-
Duodenal d1 ulcer	10	34.5
Mallory weiss tear	-	-
Large hiatus hernia	-	-

The above data is pictured in next graph.

Figure 2:Characteristic of ODG scopy results of both control and inverteniional group participants



Discusion:

Human upper gastrointestinal tract illnesses span a wide spectrum disease due to pylori invasion. Sadly, eradication therapy does not always work and may possibly have adverse effects[5]..Helicobacter pylori eradication failures reports are drastically increasing now-a-days[6].Therefore, a continuous search for new therapeutic approaches to treat H. pylori infection is needed, with the best first-line treatment also considered the best "rescue therapy" for such a common infection[7]. A recent study in Japan showed that the eradication rate of clarithromycin-resistant strains was only 7 %, while the eradication rate of clarithromycin-susceptible strains was 89.6%[8]. Resistance to metronidazole is also high, ranging from 0%to70%worldwide[9].Although tetracycline resistance is generally minimal, its usage is constrained by adverse effects . Levofloxacin is effective against Helicobacter. pylori, but its use is limited due to the development of resistance[10].Amoxicillin is less resistant and remains an effective antibiotic for H. pylori(11).However, because to the rise of drug-resistant strains, the percentage of H. pylori eradication has dropped to less than 80% in recent years. Amoxicillin (AMPC), clarithromycin (CAM), metronidazole (MET), and levofloxacin (LVFX) are commonly used as part of standard antibiotic therapy. However, the rise of strains resistant to CAM, MET, and LVFX has grown to be a significant

issue .[12]The data indicate that antibiotic resistance is common, and doctors conclude that sometimes concomitant treatment with another drug may be necessary[11].

In our study, the total patients were 75 where, 51 (68%) were males and 24 (32%) were females. The male patients were found to be higher than the female patients, which is in contrast to the study conducted by Antonio Francesco Ciccaglione et al (2019)[5]. However the risk factor for H. pylori infection were gender unbiased.

Categorizing patients based on their age will help our study about their development throughout their life. From the analysis, the early adulthood (19-35 years) and adulthood (35-50 years) population in both control and interventional groups have developed the H pylori infection higher than the other populations such as late adulthood, young old and old population. The data from various sources indicated that as the age increases the risk of infection also increases.

Since illiteracy could affect the patient's understanding of the study, the educational background was studied in both the interventional and control groups and the patients who underwent primary schooling were 24.1% and 37% respectively. The Undergraduate patients were 58.6% and 56.1 % respectively.The Postgraduate patients were 17.2% and 6.5% respectively. This data indicates that the study population has underwent the school education which will help us in making them understand their conditions even better, which will further cause increase in the quality of life of patients, prevention of recurrence and also possibly prevent resistance.

In 1940, Sorensen et al. were the first to identify and isolate the iron-binding glycoprotein lactoferrin (LF) from milk[13]. The concentration of LF in human colostrum ranges from 5–8 mg/ml, whereas in mature milk it ranges from 1–3 mg/dl [14,15]. High LF concentrations in colostrum are thought to play an crucial role in infant development and infection prevention. LF is present in exocrine fluids such as tears and saliva[16] and in the second granules of polymorphonuclear leukocytes[17], and has various properties such as growth inhibition of various microorganisms, immunomodulation, anti-inflammatory effects, and cancer prevention. Effective[16,18].

A number of factors led to the selection of bLf for this investigation among the novel variations produced lately in H. pylori targeted therapy[19].
The following evidence suggests that bLf might increase H. pylori eradication rates:

- bLf **was** found to be bactericidal **against** H. pylori after 2 days **of exposure**[20].
- bLf may inhibit H. pylori from utilizing iron due to its robust iron-binding affinity[21]. The outer membranes of the bacteria may also express specific receptors for bLf to internalize the iron-saturated protein [22] in fact, other iron chelators such desferoxamine hinder H. pylori development[23].
- An additional mechanism based on the interaction of bLf with the bacterial surface may play a role. This mechanism was originally proposed to explain the bactericidal effects of bLf against S. mutans and Vibrio cholerae [24].
- It has been noted that bLf may attach to the outer membrane of Gram-negative bacteria, cause the production of lipopolysaccharides, and ultimately eradicate the bacteria by causing osmotic damage [25,26].

- Several other different possible mechanisms by which lactoferrin inhibits the growth of several microorganisms have been proposed, including microbial cell wall structural changes, complete loss of membrane potential and integrity, indirect effects on enzyme activation, increased generation of. metabolic by-products of aerobic metabolism, iron deficiency and a combination of these factors.
- In addition, pepsin-induced degradation of LF releases lactoferricin (LFcin), another potent antibacterial peptide[27,28]. Interestingly, LFcin inhibits H. pylori urease activity[27]. Production of ammonia by urease which is a factor released by H. pylori act as a critical factor that allows the bacterium to survive in the acidic environment of the gastrum[28].
- Reports also support the antiviral activity of LF. It can prevent viral entry into host cells by binding to cell surface proteoglycans, binding to viral proteins or inhibiting intracellular viral transport[30,31]. In addition to the direct effect of LF on H. pylori, its anti-inflammatory effect may also explain the therapeutic properties of LF in Helicobacter pylori-related pathology, including gastric damage[30-35].
- LF can also modulate the inflammatory response by regulating immune cell surface receptors intracellular signaling pathways and regulates inflammatory cytokine production and iron oxidation [36-39].
- In addition, evidence suggests that LF may exert anticancer effects by inhibiting cancer cell migration and proliferation and inducing apoptosis[40,41]

Based on this evidence, we considered it likely that bLf may contribute to the improvement of H. pylori eradication rates with antibiotics. In 2003, Di Mario and colleagues first reported the effect of bLf alone on H. pylori in humans.Oral administration of bLF (200 mg b.i.d) with 7-day triple eradication therapy was successful in eradicating H. pylori in 100% of patients, while triple therapy without bLF was effective in only 76.9%[42].

In a recent multicenter study, the same author **showed** that bLf is an effective **adjunct** to 7-day triple therapy **of** H. pylori eradication[4]**.** Based on these study reports, we planned to conduct a randomized controlled trail with two groups receiving respective treatments.

In accordance with our results, a meta-analysis by Zoun et al.17 showed that bLf also improves the treatment of H. pylori in an ITT analysis of data from 1343 patients in 9 randomized trials. It was also found that patients taking bovine lactoferrin had fewer drug-related side effects[43].

In our study the majority of the patients received triple therpay with the combination of amoxicillin+tinidazole+omeprazole and resulted in 76.2 % of eradication in interventional group and 61.1%in control group . In particular, Zoun et al **stated** that **amoxicillin does not increase efficacy** when combined with bLf. However, it should be noted that the only published data on the interaction between amoxicillin and bLf **are** based on **the following proposed** animal **model:** Dial et al(44).

In the open, prospective, randomized single-center study described here, it was found that the addition of bLf to the standard 1-week triple therapy against H. Pylori significantly increased the eradication rate and decreased side effects like nausea, acid reflux, abdominal pain and epigastric pain which is correlating with our study with decrease in side effects like nausea and abdominal pain[4].

Combining bLf with tinidazole, amoxicillin, and omeprazole may have a synergistic impact by attacking H. pylori from multiple angles, which could result in the total eradication of the bacterial infection.In contrast to our findings, a recent study comprising three centers and conducted by Zullo and colleagues [45], found no distinction between triple therapy as standard and triple therapy plus bLf. They specifically asserted that the combination of amoxicillin and bLf had no increased efficacy. However, it should be highlighted that Dial et al.[44]'s proposed animal models provide the basis for all published data on the interaction between amoxicillin and bLf. Although this impact was not statistically different from bLf alone, the authors of that research saw a full clearance of the bacteria while combining, amoxicillin and bLf.

Conflict of interest:
There are no conflicts of interest concerning the research,authorship and publication of this article.

Acknowledgments:
All participants who participated in the study and assisted in the research process are thanked by the author(s).

Conclusion:
The reasons for occasional failure of *H. pylori* treatment are still unclear, although bacterial resistance and poor patient compliance are believed to be the prime factors. The occurrence of mild but unpleasant side effects such as metallic taste in the mouth, diarrhea and nausea may cause the patient to interrupt the prescribed course of antibiotics, thus leading to bacterial resistance. This situation has forced the researchers to look for alternative therapy with bLf in the last 2 decades for the eradication of *H. pylori.* Based on the results obtained from our current prospective randomized interventional study data, we conclude that the addition of bLf may enhance the efficacy of proton pump based triple therapy for *H. pylori* eradication and this could be a valid alternative regimen to the standard treatment protocol. In the current study, this therapy has found to provide satisfactory eradication rates of 76.2% vs. 61.5% in interventional and control group respectively which was confirmed using urea breath test. We have also analysed the symptoms associated with *H. pylori* infection such as abdominal discomfort, epi-gastric pain, acid reflux, nausea, belching, early satiety, heart burn, bloating, regurgitation, vomiting, anorexia and indigestion using GOS, which had shown that the symptoms have significantly reduced in interventional group when compared to control group which may be due to the addition of bLf and so higher quality of life to the patients (based on symptom relief).This study demonstrated that bLf was an effective adjuvant to 14 days triple therapy for eradication of *H. pylori* infection.

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