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Randomized Controlled Trial: Adjuvant Effect of Bovine Lactoferrin with Standard triple therapy in *Helicobacter Pylori* eradication

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ABSTRACT

Background: Eradication rate of *Helicobacter pylori (H. Pyl)* is different in different geographic localities and not exceeding 70-80%. So, there is a need to identify new effective alternative therapies.

Aim: To evaluate the efficacy of adjuvant (Bovine lactoferrin) with standard triple therapy (STT) in *H. Pyl* eradication.

Methodology: Multi-centre Randomized control trial study was done. A total of 100 patients were randomized into two study groups. Group-A (n=50) participants were treated with omeprazole (20mg), clarithromycin (500mg), amoxicillin (1000mg) all twice daily (B.D) for seven days. Group-B (n=50) participants were treated with STT plus bovine lactoferrin (B.Lf) B.D for seven days.

Results: From a total of 100 patients 5 patients lost to follow up. Before therapy most prominent symptom was Epigastric pain. At fourth week of completion of therapy 73.0% patients showed negative histopathology for *H. Pyl* eradication from all groups. On analysis between groups it was found that in group-A 58.0% (29/50) patients showed eradication rate while group-B patients achieved 88.0% (44/50) success rate.

Conclusion: This randomized study demonstrated that B.Lf improved the *H. Pyl* eradication rate and could be an effective adjuvant with STT for seven days for *H. Pyl eradication.*

Keywords: *Helicobacter pylori*, Bovine lactoferrin, Standard triple therapy

INTRODUCION

Marshall and Morris discovered *Helicobacter Pylori* (*H. Pyl*) (Kusters, van Vliet et al. 2006). *H. Pyl* is a most common bacterial pathogen worldwide. *It* makes a pool of ammonia on its surface to neutralize the gastric acid which enables it to make colonies (Pal, Sanal et al. 2011). After successful colonization, *H. Pyl* resides underneath the gastric mucus sheet which has a higher pH than gastric lumen (Schreiber, Konradt et al. 2004). The prevalence rate of *H. Pyl* and its associated disorders have been highly conflicting all over the world. Previous estimation proves that 50% of world population is *H. Pyl* carrier. Prevalence of infection tends to be more in developing countries like Pakistan, Bangladesh and India etc but its incidence is declining in western countries as standards of livelihood rise.

First line therapy of one week for *H.pyl* eradication according to Maastricht Consensus Report is standard triple therapy (STT). The therapy includes proton pump inhibitor plus two antibiotics (Malfertheiner, Megraud et al. 2012). PPI work by decreasing gastric acid secretions, because in the acidic surroundings of stomach antibiotics lose their efficacy. Clarithromycin disrupts ribosomal functions while amoxicillin destroys bacterial cell wall (Vakil and Megraud 2007). *H. Pyl* eradication rate should be greater than 80% (Egan, Katicic et al. 2007) (Tolone, Pellino et al. 2012). However, clinically eradication rates are less than 80% for many of

standard treatment regimens (Egan, Katicic et al. 2007). The main reason for treatment failures are poor patient compliance and antibiotic resistance. This critical situation has compelled researchers to look for alternative strategies to eradicate *H. Pyl* infection (de Bortoli, Leonardi et al. 2007).

B.Lf is a natural polypeptide, glycoprotein belongs to transferrin group and it transports iron even in highly acidic surroundings of stomach. B.Lf is present mainly in milk but to a lesser extent in mucosal secretions, blood plasma, pancreatic and seminal fluids. In humans bovine specific granules of the polymorphonuclear appears to be a leading factor in the host's protection against *H. Pyl* (de Bortoli, Leonardi et al. 2007);(Di Mario, Aragona et al. 2006);(Okuda, Nakazawa et al. 2005). Ingestion of B.Lf has been reported to show anti-infective, anti-cancer, and anti-inflammatory effects (Yamauchi, Wakabayashi et al. 2006). *H. Pyl* utilizes iron for its growth and development whereas B.Lf binds with iron with great affinity. So, in this way, B.Lf prevents *H. Pyl* growth. It also plays a key role in suppression of *H. Pyl* infection by inhibiting its affection to gastric epithelial surface (Di Mario, Aragona et al. 2006);(Senkovich, Ceaser et al. 2010).

The rational of our study was to compare the efficacy of adjuvant (B.Lf 250mg) with one week STT in *H. Pyl* eradication. In our setup, STT is most commonly used for eradication of *H. Pyl* and data is lacking in Pakistan regarding use of STT plus B.Lf for *H. Pyl* eradication. So, this study helped to see the effectiveness of this therapy.

MATERIALS AND METHODS

A Total of 100 patients were enrolled in the study who visited outpatient department of Medicine on the basis of age 15-55 years of both genders. They were labeled *H. Pyl* infected patients on histopathology of the biopsy sample taken during endoscopy. On the other hand patients who had history of previously failed *H. Pyl* eradication therapy, acid lowering surgery, any past history of upper GIT (esophageal, gastric or duodenal) surgery, patients on treatment with PPI within the last two weeks or any antibiotics within the last four weeks before participation in the study, allergy to clarithromycin, pregnant or lactating women, subjects with hepatic and renal functions impairments and any neoplasm defined by history were excluded from the research.

Study Design

Multi-centre Randomized controlled trial study was conducted. After taking informed consent a total of 100 patients fulfilling the inclusion and exclusion criteria were enrolled from outpatient Medicine department. Subjects were randomized into two arms (given below) using block randomization. A block size of 25 randomly determined to avoid selection bias. For each centre two blocks were used to generate the random numbers. Randomization was central. Group-A was given STT including Omeprazole 20mg B.D, Amoxicillin 1000mg B.D and Clarithromycin 500mg B.D all for one week. While Group-B received STT plus B.Lf 250mg B.D also for one week. Study subjects were advised to account of any adverse effects. At the end of study, all patients, regardless of the endoscopic findings (Gastritis, ulceration), underwent the same one week protocol of eradication therapy. *H. Pyl* eradication was confirmed on negative endoscopy based histopathology results at 4-6 week. Their demographic data like name, age, gender, address, S.E.S, smoking status and BMI were noted. WHO classification was used to evaluate BMI. While S.E.S status was defined on basis of lowest wages (10,000 Rupees) of a laborer as recommended by government was taken as cut off value**.**

DATA ANALYSIS

All the collected data was entered and analyzed by using computer software SPSS version 20.0. Qualitative data like gender, SES, education, smoking status was calculated in the form of frequency and percentages. All quantitative data like age, BMI were presented in the form of mean ± S.D. Pearson chi-square test was used to compare the efficacy of groups. P-value < 0.05 was considered as statistically significant.

RESULTS

Out of 100, 95 patients completed the study. No significant difference was observed among two groups for gender, age, BMI, S.E.S and smoking status (Table-1). A total of 50 patients were in Group-A (24 male and 26 female) having mean age of 34.16 ± 11.43 years. In Group-B, there were also 50 patients (31 male and 19 female) with mean age of 34.94 ± 12.28 years.

As far as eradication was concerned, it was found that in Group-A 58.0% (29/50) patients showed negative histopathology while in Group-B 88.0% (44/50) patients showed *H. Pyl* eradication rate. Eradication rate was significantly higher in Group-B as compared to Group-A (P = 0.002). There was no statistical significant influence of gender was seen on eradication (P > 0.05). Before Therapy Major presented symptoms were Epigastric pain and Heartburn. In the study, three patients in Group-A and two patients from Group-B lost post therapy follow up.

Table-1 Demographic Characteristics of *H. Pyl* positive patients who were randomly assigned into Group-A and Group-B

Variables		Study Groups		
		Group-A (STT)	Group-B (STT + B.Lf)	P-value
Gender	Male	24(48.0%)	31(62.0%)	0.159
	Female	26(52.0%)	19 (38.0%)	
Smokers	Yes	32(64.0%)	29(58.0%)	0.341
	No	18(36.0%)	21(42.0%)	
S.E.S	Lower Class	32(64.0%)	36(72.0%)	0.391
	Middle Class	18(36.0%)	14 (28.0%)	
Age		34.16±11.43	34.94±12.28	0.204
BMI		25.76±3.07	25.01±4.54	0.243

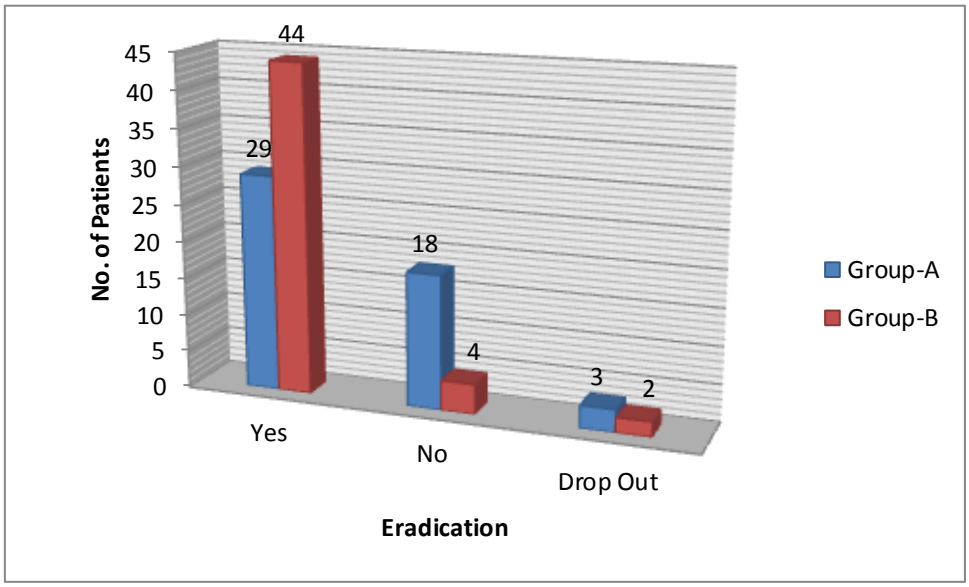

Figure-1 Graphical Distribution of *H. Pyl* Eradication with Respect to Study Groups

DISCUSSION

In the study 54.5% males and 45.5% females. were found infected with *H. Pyl*. There are many studies revealing strong association of *H. Pyl* infection with gender. Different researchers confirmed in their studies that the *H. Pyl* infection was quite predominant in male which is in accordance with our collected results (de Martel and Parsonnet 2006); (Jafarzadeh, Ahmedi-Kahanali et al. 2007); (Valliani, Khan et al. 2013). But results of our clinical study are inconsistent with (Kanbay, Gur et al. 2005), in which 60.6% were females and 42.9% were males.

Significant reason for the reduced success of STT is the mounting rate of *H. Pyl* infection towards antibiotics resistance (Jafri, Hornung et al. 2008). Worldwide carithromycin resistance is rising and *H. Pyl* treatment failure is foremost increasing problem (Mollison, Stingemore et al. 2000). Therefore, we need to pay attention to identify adjuvants and also by clever means to combine them with on-going standard therapies to increase the *H. Pyl* eradication rate. As a need of time, researchers focused on the potential role of B.Lf in therapy of various gastrointestinal disorders (Zou, Dong et al. 2009).

In our study *H. Pyl* eradication was 88.0% in group-B patients in which B.Lf was added with STT. However, a study suggested that use of B.LF as an adjuvant with STT (clarithromycin, tinidazole and rabeprazole) for one week showed significantly increased eradication rate (92.2%) as compared to STT alone (71.2%). B.Lf was found effective in this study (Di Mario, Aragona et al. 2003). A study was also conducted, to see that either by adding an adjuvant with STT (esomeprazole, tinidazole and clarithromycin) could be valuable in enhancing its effectiveness in the eradication of *H. Pyl* infection (Di Mario, Aragona et al. 2006). Maximum achieved eradication rate was found 93.0%. After completion of therapy, they suggested that the use of B.Lf could be a new agent to support the antimicrobials in the eradication of *H. Pyl* (Di Mario, Aragona et al. 2006). B.Lf is one of the antimicrobial proteins. It interacts with cellular

lactoferrin receptors that play a significant role for its antimicrobial activity. It possesses potential anti-bacterial, anti-fungal, anti-viral and anti-parasitic activities. B.Lf with STT improves the eradication rate of the bacteria (*H. Pyl*). Iron sequestration, membrane destabilization and targeting of bacterial virulence are mechanisms through which the protein exercises its antibacterial activity towards different bacterial pathogens. Iron is one of the necessary nutritional requirements for most bacteria. The binding property of lactoferrin with iron contributes to its antibacterial activity, by sequestering bacterial iron. Iron sequestration is a significant antibacterial action of lactoferrin supported by different studies. Thus in this way it inhibits the growth of broad spectrum bacterial strains (Marr, Jenssen et al. 2009).

A study reported cure rate regarding eradication of *H. Pyl* infection. It revealed that the bacteria was eradicated in 73/101 patients from group-A (esomeprazole, clarithromycin, amoxicillin) and in 93/105 from group-B (STT + B.Lf + Probiotic). Whereas, results for groups-A and group-B were 76% and 92.1% respectively. Group-B had higher cure rate as in it B.Lf and probiotic cover was given additionally (de Bortoli, Leonardi et al. 2007). Various studies are under way to evaluate new antibiotics and different therapeutic schedules to increase the efficacy of *H. Pyl* eradication therapy. Zou, Dong et al. (2009) findings are also consistent with above mentioned studies and also supports our study results to a greater extent.

This study suggested that adjuvant use of B.Lf with STT provided improve eradication rate and therefore, it could be a novel agent in assisting antimicrobial actions. The results of study should have clinical implications and to set trend for further research also.

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