Lactoferrin as an added therapy in the treatment of *Helicobacter pylori*

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Background
Bovine lactoferrin (bLF), an iron-binding glycoprotein, is a nonenzymatic antioxidant found in the whey fraction of fermented milk as well as in colostrum. The possibility that bLF may help improve the *Helicobacter pylori* eradication rate was first conceived in 1997 when an in-vitro study by Yamazaki and colleagues showed that bLF was bactericidal to *H. pylori* in *Brucella* broth. Later in-vitro studies have confirmed the same and yielded evidence of the possible mechanism of the bactericidal action of bLF, relating it to the high iron-binding affinity and prevention of iron utilization by *H. pylori*. Lactoferrin is used for treating stomach and intestinal ulcers, diarrhea, and hepatitis C.

Aim
The aim of the study was to verify the value of adding lactoferrin to the treatment of *H. pylori* infection.

Patients and methods
This study was conducted on 50 patients selected from Internal Medicine Department, Benha University Hospital. They were divided into group 1, which included 25 patients who were treated with traditional therapy (clarithromycin, omeprazole, amoxicillin, or metronidazole), and group 2, who were treated by traditional therapy plus lactoferrin (pravotin) for 1 week. *H. pylori* stool antigen testing was performed for both groups before and after therapy to assess the response to therapy. Results were statistically analyzed.

Results
Our results show that addition of lactoferrin improves the *H. pylori* infection eradication rate.

Keywords:
Helicobacter pylori infection, lactoferrin, stool antigen, traditional therapy

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Introduction

Helicobacter pylori is a Gram-negative, spiral bacterium that locates itself on the epithelial surface of the stomach. It is thought to be the most common bacterial infection worldwide. Virtually all persons infected by this organism develop gastritis, a signature feature of which is the capacity to persist for decades, leading to chronic inflammation of the underlying mucosa. It has been recognized to be associated with increased risk for chronic gastritis, peptic ulcer disease (PUD) (gastric and duodenal), gastric mucosal-associated lymphoid tissue lymphoma, and gastric adenocarcinoma. The WHO has described *H. pylori* as a class 1 carcinogen for gastric carcinoma. Isolation of the organism by Warren and colleagues in 1983 has modified the management of PUD [1].

Eradication of *H. pylori* is considered a necessary step in the management of these diseases. First-line traditional therapy eradication regimens (proton pump inhibitor plus clarithromycin and amoxicillin or metronidazole) are inconvenient and achieve unpredictable and often poor results. Second-line therapy includes traditional therapy plus lactoferrin [2].

Bovine lactoferrin (bLF), an iron-binding glycoprotein, is a nonenzymatic antioxidant found in the whey fraction of fermented milk as well as in colostrum. The possibility that bLF may help improve the *H. pylori* eradication rate was first conceived in 1997 when an in-vitro study by Yamazaki *et al.* [3] showed that bLF was found to be bactericidal to *H. pylori* in *Brucella* broth. Later in-vitro studies have confirmed the same and yielded evidence of the possible mechanism of the bactericidal action of bLF, relating it to the high iron-binding affinity and prevention of iron utilization by *H. pylori* [4].

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Lactoferrin is used for treating stomach and intestinal ulcers, diarrhea, and hepatitis C. It is also used as an antioxidant and to protect against bacterial and viral infections. Other uses include stimulating the immune system, promoting healthy intestinal bacteria, preventing cancer, and regulating the way the body processes iron [5].

Patients and methods

Inclusion criteria

Patients of both sexes who were aged 12–60 years and positive for *H. pylori* antigen in their stool were eligible for participation in this study.

Exclusion criteria

Patients with GIT malignancy, those with iron deficiency anemia, and patients negative for *H. pylori* antigen in stool were exempted from the study.

All patients were subjected to the following: history taking, including age, sex, occupation, address, marital status, habits, past history of any disease, family history, and any present complaint; and clinical examination, including general and systemic examination.

Laboratory investigation

Complete blood count, liver function tests [alanine aminotransferase (ALT), aspartate aminotransferase, and bilirubin], and *H. pylori* antigen test in stool samples (before and after treatment) were conducted.

The clinical data were recorded on a report form. These data were tabulated and analyzed using the computer program statistical package for social science (SPSS, version 16; SPSS Inc., Chicago, Illinois, USA) to obtain the following.

Statistical analysis

Descriptive data

Descriptive statistics were calculated for the data in the form of mean and SD for quantitative data and frequency and distribution for qualitative data.

Analytical statistics

In the statistical comparison between the different groups, the significance of difference was tested using one of the following tests: the paired *t*-test, which was used to compare the mean of variables before and after treatment in the same group; and the Student *t*-test, which was used to compare the mean of two groups of quantitative data.

$$t = \frac{\overline{x}_1 - \overline{x}_2}{\sqrt{\frac{SD_1^2}{n_1} + \frac{SD_2^2}{n_2}}}.$$

Intergroup comparison of categorical data was performed using the χ^2-test.

$$\chi^2 = \frac{\sum(\text{observed} - \text{expected})^2}{\text{Expected}},$$

$$\text{Expected} = \frac{\text{Column total} \times \text{row total}}{\text{Grand total}}.$$

A *P* value less than 0.05 was considered statistically significant, whereas values more than 0.05 were considered statistically insignificant and values less than 0.01 were considered highly significant in all analyses.

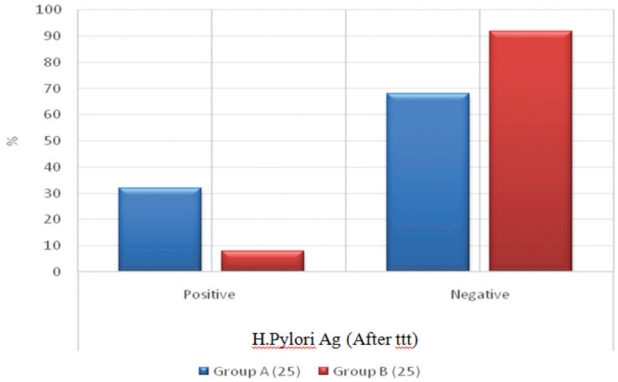
Results

The comparison between the studied groups before treatment showed no significant statistical values, except for the ALT level. The comparison between the studied groups after treatment showed significant statistical values for hemoglobin level, mean corpuscular volume (MCV) level, ALT level, and *H. pylori* antigen in stool (Table 1 and Fig. 1).

Discussion

bLF, an iron-binding glycoprotein, is a nonenzymatic antioxidant found in the whey fraction of fermented milk as well as in colostrum. The possibility that bLF may help to improve the *H. pylori* eradication rate was first conceived in 1997 when an in-vitro study by Yamazaki *et al.* found bLF to be bactericidal to *H. pylori* in *Brucella* broth. Later in-vitro studies have confirmed the same and yielded evidence of the possible mechanism of the bactericidal action of bLF, relating it to the high iron-binding affinity and prevention of iron utilization by *H. pylori* [4].

Figure 1



Comparison between the studied groups after treatment showing significant statistical values for *Helicobacter pylori* antigen in stool samples

Table 1 Comparison between the studied groups after treatment showing significant statistical values for *Helicobacter pylori* antigen in stool samples

	Group A (*n*=25) (mean±SD)	Group B (*n*=25) (mean±SD)	Test	*P* value
Age (years)	34.0±16.17	31.92±15.05	0.47#	0.64
Sex [*n* (%)]				
Male	14 (56.0)	18 (72.0)	1.39^	0.24
Female	11 (44.0)	7 (28.0)		
CBC (before treatment)				
Hb (g/dl)				
Abnormal	–	–	1.08#	0.29#
Normal	–	–	–	–
Mean±SD	12.69±2.01	13.28±1.86		
MCV				
Abnormal	7 (28.0)	6 (24.0)	0.10^	0.75
Normal	18 (72.0)	19 (76.0)	1.54#	0.13
Mean±SD	80.41±6.75	83.30±6.56		
Liver function tests (before treatment)				
AST	26.44±3.98	25.32±5.62	0.813#	0.42
ALT	20.72±3.70	18.28±4.13	2.20#	0.03*
Bilirubin	0.68±0.11	0.65±0.10	0.93#	0.36
Helicobacter pylori Ag (before treatment)				
Positive	25 (100)	25 (100)	–	–
Negative	0 (0.0)	0 (0.0)		
CBC (after treatment)				
Hb (g/dl)	12.70±1.96	14.22±1.82	2.85#	<0.01**
MCV	80.39±6.75	87.58±6.35	3.88#	<0.01**
Liver function tests (after treatment)				
AST	26.40±3.95	25.28±5.51	0.83#	0.41
ALT	20.76±3.60	18.24±4.12	2.31#	0.03*
Bilirubin	0.67±0.11	0.64±0.12	0.97#	0.34
Helicobacter pylori Ag (after treatment)				
Positive	8 (32.0)	2 (8.0)	4.50^	0.03*
Negative	17 (68.0)	23 (92.0)		

Ag, antigen; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CBC, complete blood count; Hb, hemoglobin. #Student *t* test. ^Chi square test. *Statistically significant. **Highly significant.

Lactoferrin is used for treating stomach and intestinal ulcers and diarrhea. It is also used as an antioxidant and to protect against bacterial and viral infections. Other uses include stimulating the immune system, promoting healthy intestinal bacteria, preventing cancer, and regulating the way the body processes iron [5].

H. pylori is a Gram-negative, spiral bacterium that locates itself on the epithelial surface of the stomach. It is thought to be the most common bacterial infection worldwide. Virtually all persons infected with this organism develop gastritis, a signature feature of which is the capacity to persist for decades, leading to chronic inflammation of the underlying mucosa. It has been recognized to be associated with increased risk for chronic gastritis, PUD (gastric and duodenal), gastric mucosal-associated lymphoid tissue lymphoma, and gastric adenocarcinoma. The WHO has described *H. pylori* as a class 1 carcinogen for gastric carcinoma. Isolation of the organism by Warren and colleagues in 1983 has modified the management of PUD [1].

Eradication of *H. pylori* is considered a necessary step in the management of these diseases. First-line traditional therapy eradication regimens (proton pump inhibitor plus clarithromycin and amoxicillin or metronidazole) are inconvenient and achieve unpredictable and often poor results. Second-line therapy may include traditional therapy plus lactoferrin [2].

In the present study, the results show that addition of lactoferrin improves the *H. pylori* infection eradication rate.

In agreement with our results was that of di Mario *et al.* [6], who found that the addition of 200 mg of bLF twice a day for 7-day triple therapy had a superior eradication rate (92%) in comparison with the standard 7- or 10-day triple therapy (71 and 72%, respectively) using rabeprazole (20 mg twice daily), clarithromycin (500 mg twice daily), and tinidazole (500 mg twice daily) [6].

Similarly, Tursi *et al.* [7] reported that quadruple therapy is now recommended as a second-line therapy for

H. pylori infection. It has been found that quadruple therapy is very effective in retreating *H. pylori*-positive patients with a failure of a first attempt to cure this infection, especially when *Lactobacillus casei* is added [7].

The present study as well agrees with that of Zou *et al.* [8], who mentioned that bLF improves the side effects and increases eradication rates when associated with triple therapy. It is possible that the combination of bLF with clarithromycin, amoxicillin, and esomeprazole provokes a synergistic effect by launching attacks from different directions against *H. pylori* that could lead to a better complete clearance of the bacterial infection [8].

Okuda *et al.* [9] proved that bLF-based therapy could potentially improve *H. pylori* eradication rates by ~10% without any significant impact on the treatment-associated adverse effects.

Ellison [10] found that lactoferrin appears to be an important factor in the host's defense against a wide range of bacteria. Potential mechanisms through which bLF inhibits the growth of certain microorganisms (including *H. pylori*) have been hypothesized. These include antimicrobial activity (bacteriostatic and bactericidal effects), structural changes in the microbial cell wall, complete loss of membrane potential and integrity, indirect effects on enzyme activation, increased generation of the metabolic by-products of anaerobic metabolism, iron deprivation, and a combination of these factors [10].

In contrast to our results, Zullo *et al.* [11] reported no difference between standard triple therapy and triple therapy+bLF in a recent study performed in three centers.

Opekum *et al.* [12] also found that lactoferrin is not efficacious against *H. pylori* infection as a single agent but must be added to the triple therapy to improve the result.

In particular, they claimed that there was no enhanced efficacy when amoxicillin was combined with bLF. However, it should be noted that the only published data on the interaction between amoxicillin and bLF are based on animal models proposed by Dial *et al.* [13].

In conclusion, our results showed that addition of lactoferrin can improve the *H. pylori* infection eradication rate and the present study recommends adding bLF to traditional therapy eradication regimens.

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Conflicts of interest
There are no conflicts of interest.

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