The impact of bismuth addition to sequential treatment on *Helicobacter pylori* eradication: A pilot study

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ABSTRACT

The success of the current anti-*Helicobacter pylori* (*H. pylori*) treatment protocols is reported to decrease by years, and research is needed to strengthen the *H. pylori* eradication treatment. Sequential treatment (ST), one of the treatment modalities for H. pylori eradication, includes amoxicillin 1 gr b.i.d and proton pump inhibitor b.i.d. for first 5 days and then includes clarithromycin 500 mg b.i.d. metronidazole 500 mg b.i.d. and a proton pump inhibitor b.i.d. for remaining 5 days. In this study, we investigated efficacy and tolerability of bismuth addition in to ST. We included patients that underwent upper gastrointestinal endoscopy in which *H. pylori* infection was diagnosed by histological examination of antral and corporal gastric mucosa biopsy. Participants were randomly administered ST or bismuth containing ST (BST) protocols for the first-line *H. pylori* eradication therapy. Participants have been tested by urea breath test for eradication success 6 weeks after the completion of treatment. One hundred and fifty patients (93 female, 57 male) were enrolled. There were no significant differences in eradication rates for both intention to treat population (70.2%, 95% confidence interval [CI]: 66.3-74.1% vs. 71.8%, 95% CI: 61.8-81.7%, for ST and BST, respectively, $p > 0.05$) and per protocol population (74.6%, 95% CI: 63.2-85.8% vs. 73.7%, 95% CI: 63.9-83.5% for ST and BST, respectively, $p > 0.05$). Despite the undeniable effect of bismuth, there may be several possible reasons of unsatisfactory eradication success. Drug administration time, co-administration of other drugs, possible *H. pylori* resistance to bismuth may affect the eradication success. The addition of bismuth subcitrate to ST regimen does not provide significant increase in eradication rates.

KEY WORDS: Amoxicillin; metronidazole; clarithromycin; antibiotic resistance; *Helicobacter pylori*
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INTRODUCTION

Successful treatment of *Helicobacter pylori* (*H. pylori*) generally requires the use of several antibacterial agents simultaneously. With the exception of a limited number of studies, increasing clarithromycin resistance causes the decline in effectiveness of the legacy triple therapy (i.e., a proton pump inhibitor [PPI], clarithromycin and amoxicillin) and intention to treat (ITT) eradication rates have been reported lower than 80% (grade F) in several studies [1].

The impact of resistance can be minimized by addition of a second, third or even the fourth drug for killing the few remaining organisms or administrating drugs in different time intervals leading to a high cure rate. With the decrease in the success of standard triple treatment, new treatment protocols have been developed. Sequential therapy (ST) (amoxicillin 1 g plus a PPI b.i.d. for 5 days, then clarithromycin 500 mg and tinidazole or metronidazole 500 mg b.i.d plus a PPI b.i.d for remaining 5 days) has been developed a decade ago as one of the promising therapeutic approach to solve this problem [2] and has been shown to be superior to legacy triple therapy even if there has been clarithromycin resistance [1]. However, up to now, reported cure rates of the original ST did not exceed above Grade B (90-95%) in ITT analysis. Moreover, recent studies from different countries have reported disappointing results with ST [3-6]. It is of interest whether additional changes in ST may cause further improvement in eradication rates or not.

Bismuth is cytoprotective and antibacterial agent which has been safely used for three centuries. The addition of bismuth compounds to different antibiotic combinations have been reported to provide a favorable effect on eradication rates [7-9]. There is no data on eradication rates with original ST plus bismuth in the literature.

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We aimed to compare eradication rates of ST with and without bismuth as a first-line therapy.

MATERIALS AND METHODS

Subjects

This prospective, single-center study that was performed at the Gastroenterology Department of Kecioren Education and Research Hospital in Turkey in 2013. This trial was approved by Institutional Review Board.

Between February 2013 and July 2013, patients with dyspepsia were considered eligible for the study if they underwent upper gastrointestinal endoscopy, and *H. pylori* infection was diagnosed through histologic examination (Giemsa stain) of antral and body biopsy samples diagnosed histopathologically documented *H. pylori* infection. Patients were considered infected by *H. pylori* if resulted positive. Exclusion criteria included (1) previous attempt of *H. pylori* eradication therapy; (2) recent use of antibiotic or bismuth salts or proton-pump inhibitors in the last 2 months before the study, (3) chronic use of nonsteroidal anti-inflammatory drugs or corticosteroids, (4) severe comorbid diseases, (5) gastric malignancy including adenocarcinoma and lymphoma; (6) pregnancy or lactation, (7) diarrhea, (8) prior gastric surgery, and (9) allergy to any of the drugs in the current treatment; (10) age under 18 years. Informed consent was obtained from each patient before enrolling into the study.

The patients were randomly divided into two groups. While ST group were given rabeprazole 20 mg (b.i.d, 30 minutes before meals) and amoxicillin 1000 mg (b.i.d, an hour after meals) for the first 5 days of the treatment period and then rabeprazole 20 mg (b.i.d, 30 minutes before meals) clarithromycin 500 mg (b.i.d, an hour after meals) and metronidazole 500 mg (b.i.d, an hour after meals) in the remaining 5 days, bismuth plus ST (B+ST) group were given colloidal bismuth subcitrate 300 mg (equivalent to Bi_2O_3 120 mg; two swallowed tablets, an hour before breakfast and dinner) for 10 days with the original ST. All patients continued rabeprazole 20 mg daily during the subsequent 30 days.

The detailed written treatment protocol was given to all patients to prevent misusage medications. Compliance was defined as being taken prescribed drugs more than 90%. They were actively interviewed about side effects and compliance using a structured questionnaire 1-week after the end of the treatment.

The primary endpoint of this study was to evaluate the eradication rate of 10 days bismuth containing ST (BST) therapy. Eradication rate was measured by urea breath test (UBT) at least 6 weeks after the end of the treatment. The C14-UBT was used for this purpose. Patients were informed about not using PPI, H2 receptor blocker, antibiotic and analgesic within 1-week before to UBT in order to avoid false negativity. After an overnight fasting, 37 kBq (1 mCi) of C14 urea/citric acid composition (Helicap, Noster System AB, Stockholm, Sweden) with 250 mL of water was given to patients orally. Breath samples were collected with a dry cartridge system (Heliprobe Breath Card, Noster System AB). A small desktop Geiger–Muller counter (Heliprobe analyzer, Noster System AB) was used for the analysis. Results were given both as counts per minute (CPM) and as grade (0: Not infected, CPM < 25; 1: Equivocal, CPM 25-50; 2: Infected, CPM > 50), as suggested by the manufacturer. Patients who remained positive for *H. pylori* after the initial treatment as determined by the C14 UBT were retreated with another rescue regimen.

Statistical analysis

Both "per protocol" (PP) (excluded patients with poor compliance of therapy and patients with unavailable data after therapy) and "ITT" (included all eligible patients enrolled into the study regardless of compliance with the study protocol; patients with unavailable data are assumed to have been unsuccessfully treated) analyses were used to evaluate *H. pylori* eradication rate. Data analysis was performed using Statistical Software Package Program (SPSS) version 16.0 (IBM, USA). Intergroup comparisons of categorical variables were done using Chi-square test and continuous variables were compared using Student's *t*-test. Categorical variables were presented as percentages or counts and continuous variables were presented as mean and standard deviation in descriptive analysis. Results were evaluated at 95% of confidence interval (CI), and significance was evaluated for each parameter at $p < 0.05$.

RESULTS

Treatment groups

A total of 150 patients were included in the ITT population (ST group, n = 75; B+ST group, n = 75). Table 1 shows baseline characteristics of the two ITT populations. Groups did not differ with regard to demographical and clinical characteristics. Five patients were noncompliant to treatment protocol because of side effects. Thus, PP population consisted of 145 patients: Sequential therapy group, n = 73; bismuth plus ST group, n = 72. Major side effects were vomiting (n = 3) and abdominal pain (n = 2) (Table 2). Because of small case count per cells, statistical analysis could not be made in relation to side effects between groups.

Treatment success

Table 3 shows treatment successes of the two ITT and PP populations. The two treatments groups did not differ with

regard to *H. pylori* eradication rate for both ITT population (70.2%, 95% of CI: 66.3-74.1% vs. 71.8%, 95% of CI: 61.8-81.7%, for ST and B+ST, respectively, $p > 0.05$) and PP population (74.6%, 95% of CI: 63.2-85.8% vs. 73.7%, 95% of CI: 63.9-83.5% for ST and B+ST, respectively, $p > 0.05$).

DISCUSSION

The result of our study indicated that the success of *H. pylori* eradication by using ST regimen with or without bismuth was not satisfactory in our region. In addition, it showed that addition of bismuth subcitrate to the ST regimen did not provide a significant increase in eradication rates.

The major factor affecting the success rate of *H. pylori* eradication therapy is resistance to antibiotics. Resistance rate for clarithromycin has been reported as 8-30% and for metronidazole as 15-66% in the world [10]. Amoxicillin resistance has been reported as under %1. Since the presence of dual resistance theoretically removes both clarithromycin and metronidazole leaving only the PPI + amoxicillin dual regimen. Fourteen-day dual therapy with standard dose PPI provides approximately 50% of treatment success and approximately one-half that at 7 days [11]. Therefore, ST is likely to be an unacceptable choice in regions where both clarithromycin and metronidazole resistance are common. Recently, in our region, metronidazole resistance was reported to be more than 40% and clarithromycin resistance was reported to be more than 30% [12-14]. So that ST is also not suitable treatment protocol for our region. Previous studies from our country are in accordance with our results [15-16].

There is growing data about useful effects of bismuth compounds in *H. pylori* eradication treatment. Bismuth containing quadruple treatment has been showed to provide better eradication rates comparing to standard triple treatment [9], and it has been recommended as a first-line treatment in regions which have high clarithromycin rates [17]. However, it is unclear whether the addition of bismuth to all protocols in any way increases the eradication rate invariably or not. There is some unconvincing data in the literature. One of these studies from our country which is conducted by Ergül et al., have reported high eradication rates (90.7%) with the addition of bismuth to the standard triple regimen. However, they had not compared the same regimen without bismuth simultaneously [8]. They have compared their results with previously reported data and showed a spectacular increase (nearly 30%) in eradication rates. If these results were applicable generally, the eradication problem would have been solved by the addition of bismuth without the need for various antibiotic combinations. In addition, it has been known that success of *H. pylori* eradication varies according to race, region, virulence and impact of bacteria [18]. All these factors affect the homogeneity of the results. In another study from Turkey, Songür et al. [19] have showed no significant differences in eradication rates between protocols which include lansoprazole, tetracycline, and metronidazole with or without bismuth.

Besides the PPI-based treatments which contain PPI, amoxicillin, clarithromycin and bismuth, ranitidine bismuth based treatments which contain ranitidine bismuth, amoxicillin, and clarithromycin have been also reported not to provide sufficient success [20,21].

In our study, we did not found a significant difference in eradication rates between the protocols with and without bismuth. Despite the undeniable effect of bismuth, there may be several possible reasons of unsatisfactory eradication success. Administration time of drugs is a very important factor, which influence the effectiveness. Bismuth has been reported to facilitate the passage of the antibiotic into the microorganism. Coadministration of bismuth compounds with antibiotics has been demonstrated to reduce antibiotic resistance or increase the eradication rates of antibiotic resistant strains [22]. In an *in vitro* study, bismuth has been found to act as synergistic effect with metronidazole and clarithromycin against antibiotic resistant *H. pylori* strains in agar dilution or E-test methodology using time-kill method [23]. A simple addition

TABLE 1. Baseline characteristics of the treatment groups (intention to treat population)

Parameters	ST	B+ST	*p* value
Number of patient	75	75	
Mean age (years)	44.8±12.7	40.8±11.9	0.075
Sex (*n*, %)			
Female	45 (60)	48 (64)	0.453
Male	30 (40)	27 (36)	
Endoscopic findings (*n*, %)			
NUD	69 (92)	67 (89.3)	0.420
GU	0	3 (4)	
DU	6 (8)	5 (6.7)	0.632

Data are presented as number (%) and mean±SD. ST: Sequential treatment; B+ST: Bismuth plus sequential treatment; NUD: Non-ulcer dyspepsia; GU: Gastric ulcer; DU: Duodenal ulcer

TABLE 2. Frequency of adverse effects in both treatment groups

Parameters	ST (%)	B+ST (%)
Vomiting	2 (2.6)	1 (1.3)
Abdominal pain	1 (1.3)	1 (1.3)

Data are presented as number (%). ST: Sequential treatment; B+ST: Bismuth plus sequential treatment

TABLE 3. Treatment success of the two treatments

Parameters	ST	B+ST	*p* value
Eradication rates			
PP (%, 95% CI)	74.6 (63.2-85.8%)	73.7 (63.9-83.5%)	0.850
ITT (%, 95% CI)	70.2 (66.3-74.1%)	71.8 (61.8-81.7%)	0.873

Data are presented as number (%). ST: Sequential treatment; B+ST: Bismuth plus sequential treatment; PP: Per protocol; ITT: Intention to treatment; CI: Confidence interval

of bismuth compound to an antibiotic has been suggested not to overcome the established antibiotic resistance [24,25]. However in the most applications, using of bismuth has been recommended before meal because of it acts at low pH and using of antibiotics have been recommended after meal because of their dyspeptic side effects. We also recommended our patients to use bismuth before meal. Since coadministration is more effective than simple addition, we may fail to demonstrate the real effect of bismuth.

Bismuth salts are compounds that act topically. Less than 1% of the oral dose of commonly used bismuth compounds is absorbed. Its effect is pH dependent. In acidic pH, it is insoluble and forms complex polymers [26]. In this form, it is cytoprotective, it accumulates in ulcer crater and heal ulcer by preventing acid influx. It has also antibacterial effect by inhibiting of various enzymes produced by *H. pylori* - including urease, catalase, and lipase/phospholipase-; adhesion of *H. pylori* to surface epithelial cells; ATP synthesis; cell wall synthesis and membrane function [27,28]. For these effects bismuth must be taken by bacteria. It has been shown that bismuth enter into the *H. pylori* cell, but the mechanism of the entrance is unclear [28,29]. It has been suggested that bismuth may enter into the microorganism by diffusion or ion channel transport system [30]. In polymerized form, bismuth may not enter the microorganism and administration of bismuth in fasting condition may result in cytoprotective effect but not antibacterial effect. This practice may affect the bioavailability of the drug. In a study performed by Ciccaglione et al., bismuth had been administrated nearly after the meal time and they have shown 15% of favorable effect with addition of bismuth comparing to the same regimen without bismuth [7].

Bismuth has been suggested to damage the *H. pylori* in different ways such as destroying glycocalyx wall [28], DNA damage, competitive inhibition of bacterial enzymes. Although it has been suggested that *H. pylori* did not develop resistance against bismuth, compensatory changes have been reported in the microorganism after administration of bismuth. Nap A - a 150 kDa DNA-binding protein that protects cells from DNA damage - is found to be upregulated after administration of bismuth *in vitro* [31]. Although it has been suggested that bacteria can not gain resistance against destruction of glycocalyx wall, development of microbial resistance has been described *in vitro* for mutated microorganism which diminished production of glycocalyx [32]. Bismuth is from heavy metal class. It has been reported that bacteria could develop resistance to metals or show long-term adaptation [33].

H. pylori has also various virulence factors to cope with the human systemic and local defense mechanisms, such fighting mechanisms of the bacteria may act in bismuth treatment. *H. pylori* response against to effects of bismuth is still unclear and likely to be the subject of further studies. However, over the time bismuth-based quadruple therapy has been showed to fail showing improvement in eradication rates comparing to standard triple treatment [34].

The main limitation of the present study is the lack of any specific susceptibility testing for antibiotics. However, antibiotic susceptibility tests are not routinely performed in the first-line eradication treatment of *H. pylori* regarding our study. Our primary outcome was to investigate whether there is a beneficial effect of the addition of bismuth subcitrate to ST. This is the first study which reported the effect of original ST with bismuth in *H. pylori* eradication. Further multicenter studies are needed with large sample size and without our limitations.

In conclusion, in areas with a high prevalence of metronidazole and clarithromycin resistance, the current formulation of ST is likely to provide grade F results. Although the published studies with ST conclusively and repeatedly demonstrated its superiority to legacy triple therapy and our results (74.6%) are better than known eradication rates with triple therapy in our country (<60%). It is not a suitable choice to consistently achieve grade A results. Moreover, the addition of bismuth subcitrate did not provide a beneficial effect. Diversity in terms of drug administration or bacterial defense mechanism may have a role in this result.

DECLARTION OF INTERESTS

The authors declare no conflict of interest.

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