Background: Helicobacter pylori eradication rates have tended to decrease recently possibly related with increasing antibiotic resistance. The present study investigated the efficacy of three different ranitidine bismuth citrate (RBC) based triple regimens in a population with high prevalence of \textit{H. pylori}.

Methods: 300 consecutive \textit{H. pylori} positive patients with non-ulcer dyspepsia were randomized into three regimens: (1) RBC 400 mg, amoxicillin 1000 mg and tetracycline 500 mg [RBC-AT], (2) RBC 400 mg, amoxicillin 1000 mg and clarithromycin 500 mg [RBC-AC], (3) RBC 400 mg, metronidazole 500 mg and tetracycline 500 mg [RBC-MT]. Tetracycline was given q.i.d, all other drugs were given b.i.d. for 14 days. Gastroscopy and 14C-Urea breath test (UBT) were performed before enrollment and UBT only was repeated 6 weeks after the end of treatment.

Results: 274 patients completed the protocols. The overall ‘intention to treat’ and ‘per protocol’ \textit{H. pylori} eradication rates in all subjects were 57.6% (95% CI: 52-63) and 63.1% (95% CI: 57-68), respectively. The eradication rates achieved in the groups (RBC-AT, RBC-AC and RBC-MT) were 64.4% (95% CI: 54-74), 66.2% (95% CI: 56-76), and 58.9% (95% CI: 49-68) on ‘per protocol’ analyses, respectively. There was no difference in eradication rates, compliance and major side effects between the groups. Conclusion: The current RBC-based \textit{H. pylori} eradication therapy is not adequately effective.

Methods
Consecutive \textit{H. pylori} positive patients aged 18 years and older with non-ulcer dyspepsia were enrolled between 2003 and 2005. The presence of \textit{H. pylori} was assessed by urea breath test (UBT) and histological examination of gastric biopsy obtained at endoscopy. Subjects who tested positive for \textit{H. pylori} on both UBT and histology were included. Those who had previously received \textit{H. pylori}-eradication therapy, and those who were treated with bismuth salts, nonsteroidal anti-inflammatory drugs, PPI, H$_2$-receptor blockers, or antimicrobials within the previous 4 weeks were excluded. Other exclusion criteria were pregnancy, history of allergy to penicillin or any other antibiotic and previous gastric surgery. To make the study group more homogenous, we included only patients with non-ulcer dyspepsia. All patients provided written informed consent and the study protocol was approved by local ethic committees.

Study Design
The study was a prospective, single-center, ran-
domized, observer-blind and open trial with a parallel group design. After the selection procedure, consecutive out-patients were assigned to one of three study groups using random sampling numbers. All patients received RBC 400 mg b.i.d. along with amoxicillin 1000 mg b.i.d. and tetracycline 500 mg q.i.d. (RBC-AT protocol), or amoxicillin 1000 mg b.i.d. and clarithromycin 500 mg b.i.d. (RBC-AC protocol), or metronidazole 500 mg b.i.d. and tetracycline 500 mg q.i.d. (RBC-MT protocol), for 14 days. Side effects, if any, were scored as mild, moderate or severe according to their effect on daily activities. Patient compliance was evaluated at the end of treatment by pill count and was considered good if more than 80% of the medication had been taken. UBT was repeated 6 weeks after end of therapy. Successful eradication of bacteria was defined as negative UBT.

**Histology**

Biopsy specimens (two from the antrum and two from the corpus) were placed in 10% buffered formaldehyde and then embedded into paraffin after tissue processing. Sections were stained by hematoxylin and eosin, and with toluidine blue for *H. pylori*. The pathologist was unaware of the clinical information and UBT results. The activity of gastritis and density of *H. pylori* colonization were graded according to the Sydney classification on a scale of 0-3: none [0], mild [1], moderate [2], and severe [3].

**14C-Urea breath test (UBT)**

Antacids were stopped at least 24 h before the test, sucralfate was discontinued for 1 week before the test. After overnight fasting, patients swallowed 37 kBq (1 mCi) of an encapsulated form of $^{14}$C-urea/citric acid composition (Helicap, Noster System AB, Stockholm, Sweden) with 25 mL water. Breath samples were collected with a special dry cartridge system (Heliprobe Breath Card, Noster System AB, Stockholm, Sweden) at 10 min. Patients exhaled gently into the cartridge mouthpiece until the color of the indicator membrane changed from orange to yellow. The breathcard was inserted into a special small desktop Geiger-Müller counter (Heliprobe analyser, Noster System AB, Stockholm, Sweden) and activity counted for 250 s. Results were expressed as counts per minute (CPM), and graded (0: not infected, CPM <25; 1: equivocal, CPM 25-50; 2: infected, CPM >50) as suggested by the manufacturer. Using histology and CLO test as reference, the UBT test had been previously validated for documenting both the presence and proof of eradication of *Helicobacter pylori* in 100 patients.

**Statistical analysis**

The sample size required for the study was calculated to detect a difference of 20% in the eradication rates between the treatment protocols (by corrected chi-squared test). Based on a two-sided test, a significance level of 5%, and a power of 0.8, at least 91 patients per group were required for per protocol (PP) analysis. Assuming a dropout rate of 10%, at least 100 patients were needed to detect difference between treatment arms. Results include the binominal 95% lower and upper confidence intervals (CI).

Demographic and clinical features of the study groups were compared using Wilcoxon rank sum test. Statistical analyses of eradication rates with 95% CI were done using $\chi^2$ test with Yates' correction and the Fisher exact test. Both intention-to-treat (ITT) and PP analyses were performed. P value less than 0.05 was considered significant. All statistical analyses were performed using the statistical software Microsta and Microsoft Office Excel 2003.

**Results**

A total of 2560 patients were screened in the endoscopy unit during the study period and 300 eligible subjects were included. Thirty seven patients were not included because they had positive *H. pylori* result in only one test. Each study group consisted of 100 patients. Twenty-six patients (8.6%) dropped out of the study (Table). The demographic and clinical characteristics of the groups were comparable (Table). *H. pylori* was eradicated in 173 patients (RBC-AT n=58, RBC-AC 59, RBC-MT 56). The overall ‘ITT’ and ‘PP’ *H. pylori* eradication rate in the respective groups was 57.6% (95% CI 52-63) and 63.1% (95% CI 57-68), respectively. The eradication rates obtained in the RBC-AT, RBC-AC and RBC-MT groups were 58%, 59% and 56% on ITT analyses ($\chi^2=0.19, p=0.9$), and 64.4%, 66.2% and 58.9% on PP analyses ($\chi^2=0.14, p=0.93$). The overall PP eradication rate was similar in non-smoking patients compared to smoking patients (67.76% vs.53.84%; p=ns).

The overall prevalence of reported side effects was 9.8% in all patients. Five patients reported severe side effects requiring discontinuation of treatment (Table). Symptoms resolved in
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3-10 days. Twenty-two patients complained from mild to moderate side effects (bad taste in mouth in 7, abdominal discomfort in 7, diarrhea in 4, nausea/vomiting in 2 and headache in 2 patients); none of them required discontinuation of treatment; symptoms resolved in 1-7 days after stopping the treatment.

Discussion

The efficacy of H. pylori eradication rate with RBC-based triple regimens was poor in this study. The eradication rate was lower than 60% on ITT and less than 70% on PP analyses in all groups with an overall success rate of 57% on ITT and 63% on PP analyses. Although RBC-AC group showed a better eradication rates compared to RBC-MT group (66.2% vs. 58.9% on PP analyses; p=ns). The generally accepted minimum success rate of treatment regimens for H. pylori eradication is 80%. None of the RBC-based triple regimens reached an acceptable rate for H. pylori eradication in our study.

The poor response to H. pylori eradication regimens is most likely related to antibiotic resistance.9 Noncompliance to treatment, short therapy duration, smoking and advanced age are other factors affecting success of eradication.10 All regimens were given for two weeks and compliance rates were acceptable in all groups in our study. A poor H. pylori eradication rate has been reported in patients with non-ulcer dyspepsia compared to patients with peptic ulcer in some previous studies.11 Many studies have reported a significant rise in clarithromycin resistance in H. pylori from our population12,13 as well as from other regions.14,15 It is also well known that there is high resistance of H. pylori to metronidazole in developing countries including in our region.16 Our group has also demonstrated a recent decrease in the eradication success of combination of PPI, amoxicillin and clarithromycin when compared to its prior efficacy.17,18,19 In a survey of 128 trials done in the last 10 years using any PPI, clarithromycin and amoxicillin combination, H. pylori eradication rate reduced from about 80% between 1996-2000 to under 70% after 2000 and under 60% in 2004 and 2005.4 We did not analyze H. pylori resistance and antibiotic sensitivity in this study.

RBC-based triple regimens for H. pylori became popular since RBC, in combination with clarithromycin and metronidazole, could overcome the impact of resistance against both antimicrobials. Lopez-Brea et al showed a synergy of RBC and metronidazole combination against to metronidazole-resistant H. pylori clinical isolates.20 Midolo et al reported in vitro synergy between RBC and tetracycline or clarithromycin against H. pylori.5 RBC-based triple regimens have thus been suggested especially for regions with a high prevalence of antibiotic resistance.6,7 A well-designed Japanese study showed an increase in clarithromycin resistance related to the annual consumption of this drug.21 Tetracycline resistance in clinical isolates of Helicobacter pylori, which is associated with a nucleotide substitution in the 16S rRNA, is also well characterized and resistance rate up to 58% of Helicobacter isolates against tetracycline has been reported.22

The major limitation of our study is lack of data regarding H. pylori resistance and in vitro antibiotic sensitivity in the study population. Also,
all the study arms consisted of a regimen including RBC and did not include a PPI-based regimen for comparison. Finally, confirmation of H. pylori eradication was based solely on UBT. Despite these limitations, the present study indicates that current RBC-based H. pylori eradication regimens do not provide adequate eradication in our population and this may have implications for other developing countries as well.

References

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Received March 3, 2007. Received in final revised form July 17, 2007. Accepted August 5, 2007

Indian Journal of Gastroenterology 2007 Vol 26 July - August 177