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The efficacy of two-week therapy with ranitidine bismuth citrate, amoxicillin and clarithromycin on *Helicobacter pylori* eradication in clarithromycin-resistant and- sensitive cases

Klaritromisine dirençli ve duyarlı olgularda iki haftalık ranitidin bizmut sitrat, amoksisilin ve klaritromisin tedavisinin *Helicobacter pylori* eradikasyonundaki etkinliği

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Background/aims: *Recent studies in Turkey have documented the noticeably low success rates of H. pylori eradication. It has become more important to identify the highly successful therapy regimens since clarithromycin resistance, one of the most important factors affecting the success rate of eradication therapies, continues to increase. The aim of this study was to assess the efficacy of two-week therapy with ranitidine bismuth citrate (RBC), amoxicillin (A), and clarithromycin (C) on H. pylori eradication, and the role of C resistance in eradication.* ***Methods:*** *The study included 45 dyspeptic patients with H. pylori diagnosed by urease test and histopathological examination. C resistance was studied by real-time PCR method on antral biopsy specimens. All patients were treated with a two-week therapy consisting of RBC: 2x400 mg, A: 2x1000 mg, and C: 2x500 mg, daily. Endoscopy was repeated at least one month after the end of the treatment. Presence of H. pylori was investigated by urease test and histopathological examination on antrum and corpus biopsies. Eradication was considered when both tests were negative for H. pylori in all specimens.* ***Results:*** *Two cases were lost to follow-up. The average age of the remaining 43 patients (22 males, 21 females) was 46.3 ± 11.5 years. Mild side effects were encountered in 20 (46.5%) patients. Eradication was achieved in 35 (81.4%) patients. C resistance was studied in 26 patients and was detected in 10 (38.5%) of them. H. pylori eradication rate was 81.3% in C-sensitive (13/16) and 80% in C-resistant patients (8/10) (p>0.05).* ***Conclusion:*** *A two-week regimen of RBC-A-C is very effective for H. pylori eradication even in C-resistant patients. These results suggest that RBC-A-C combination should be preferred for H. pylori eradication in Turkey.*

Key words: Clarithromycin resistance, *Helicobacter pylori*, ranitidine bismuth citrate, treatment

Amaç: *Son yıllarda Türkiye'de yapılan çalışmalarda, H.pylori eradikasyon tedavilerinin başarısının belirgin olarak azaldığı dikkati çekmektedir. Eradikasyon tedavilerinin başarısını etkileyen en önemli faktörlerden biri olan klaritromisin direnci artmakta, bu nedenle de başarısı yüksek olan tedavilerin belirlenmesi giderek daha da önem kazanmaktadır. Bu çalışmanın amacı, iki haftalık ranitidin bizmut sitrat (RBS), amoksisilin (A) ve klaritromisin (K) tedavisinin H.pylori eradikasyonundaki etkinliğinin ve K direncinin, eradikasyondaki rolünün değerlendirilmesidir.* ***Yöntem:*** *Üreaz testi ve histopatolojik yöntemle H.pylori pozitif bulunan 45 dispeptik hasta çalışmaya alındı. K direnci, antral biyopsi örneklerinde real-time PCR yöntemi ile çalışıldı. Hastalara iki hafta süreyle, RBS: 2x400mg, A: 2x1000mg ve K: 2x500mg verildi. Tedavinin tamamlanmasından en az bir ay sonra endoskopi tekrarlandı. Antrum ve korpustan alınan biyopsilerde, üreaz testi ve histopatolojik yöntemle H.pylori araştırıldı. Tüm örneklerde her iki testin de negatif bulunduğu hastalarda, H.pylori eradikasyonun sağlandığı kabul edildi.* ***Bulgular:*** *İki hasta takipten çıktı. Kontrola gelen 43 hastanın 22'si erkek, 21'i kadın olup, yaş ortalaması 46.3 ± 11.5 idi. Yirmi (%46.5) hastada hafif yan etkiler meydana geldi. Toplam 35 hastada (%81.4) H. pylori eradikasyonu sağlandı. K direnci 26 hastada çalışıldı. Bu olgulardan 10'u (%38.5) K'e dirençli bulundu. K'e duyarlı olguların %81.3'ünde (13/16), dirençli olanların ise %80'inde (8/10) H.pylori eradike edildi (p>0.05).* ***Sonuç:*** *H.pylori eradikasyonunda iki haftalık RBS-A-K tedavisi, K direnci olan hastalarda bile oldukça etkilidir. Bu sonuç, Türkiye'de H.pylori eradikasyonunda RBS-A-K kombinasyonunun tercih edilmesi gerektiğini düşündürmektedir.*

Anahtar kelimeler: Klaritromisin direnci, *Helicobacter pylori*, ranitidin bizmut sitrat, tedavi

INTRODUCTION

Recommended treatments for first-line *Helicobacter pylori (H. pylori)* eradication are short-term proton pump inhibitor (PPI) or ranitidine bismuth citrate (RBC)-based triple therapies consisting of

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clarithromycin (C) and amoxicillin (A) or a nitroimidazole (1). However, recent studies have provided evidence that the success rates of such therapies are clearly decreasing in Turkey (2, 3).

Antibiotic resistance is one of the major factors affecting the outcome of eradication therapy for *H. pylori*. This phenomenon has been particularly important in cases infected with C-resistant *H. pylori* (4, 5). In Turkey, resistance of *H. pylori* to C is clearly increasing (6, 7). Therefore, it is very important to determine highly effective anti-*H. pylori* therapies for C-resistant patients. On the other hand, RBC has been shown to be active in vitro against both metronidazole- and C-resistant strains when given with these antibiotics (8, 9). In vivo studies have also provided evidence that RBC-based therapies are more effective than PPI-based therapies in patients with C-resistant strains of *H. pylori* (5, 10, 11).

The aim of this study was to investigate the *H. pylori* eradication rate using two-week therapy with RBC-A-C, and to assess the impact of C resistance on the efficacy of the treatment.

MATERIALS AND METHODS

Forty-five patients with dyspepsia of at least three-months' duration in whom upper endoscopy was performed and *H. pylori* infection was detected by urease test and histopathologically were included in the study. Patients with previous treatment for *H. pylori* infection, gastric resective surgery or vagotomy, gastric outlet obstruction, pregnancy, and the use of non-steroidal anti-inflammatory drugs, corticosteroids, PPIs, bismuth or antimicrobial agents in the last four weeks were excluded from the study. Further exclusion criteria were the presence of severe concurrent disease (cardiac, renal, hepatic, neurological, pulmonary, metabolic, hematological or endocrine, and suspected or confirmed malignancy), and breast feeding.

Three gastric biopsies from both antrum and corpus regions were taken and assessed for *H. pylori*. Two biopsies of each region were sent to the Pathology Department and assessed histopathologically after staining with hematoxylin-eosin and toluidine blue. The other antrum and corpus biopsies were used for rapid urease test. *H. pylori* infection was diagnosed when both urease test and histopathologic examination were positive for *H. pylori*. The patients were asked to take RBC (400 mg b.i.d) + A (1000 mg b.i.d.) + C (500 mg b.i.d.) for two weeks. At the end of the treatment, all the patients were seen and the drug boxes were monitored to determine number of pills used. Adverse events were recorded. Endoscopic examinations with three biopsies from antrum and corpus regions of the stomach were repeated at least one month after the end of the therapy. Eradication of *H. pylori* was considered when both urease test and histopathologic examination were found to be negative for *H. pylori* in all biopsy specimens. Signed consent was obtained from all participants.

C resistance was tested with real-time polymerase chain reaction (PCR) technique. C resistance mutations (A to G substitutions on the 2142nd and 2143rd nucleotide residues of 23s rRNA) were sought with TaqMan probe technology. A commercial kit was used (Roboscreen, Germany, Cat # 0204005301) with ABIPRISM 7000 (Applied Biosystems, USA) thermal cycler. Endoscopic biopsy samples were kept in phosphate-buffer-saline at -80°C until studied. DNA extraction was performed using Quiagen kit according to manufacturer's instructions; real-time PCR was also performed according to manufacturer's instructions. Briefly, 5 µl of extracted DNA, 0.5 µl of forward and reverse primers for 23s rRNA of *Hp*, 0.5 ml of mixture of TaqMan probes for A2142G and A2143G mutations and for wild type were completed to 20 µl with reaction mixture. Cycling conditions consisted of an initial denaturation at 95°C for 10 min, followed by 40 cycles with denaturation at 95°C for 30 s, annealing and extension at 60°C for 90 s with a ramping time of 20°C/s. Fluorescence radiated from TaqMan probes for the mutants or for wild type was recorded during PCR procedures. Resistant mutants or wild type bacteria were then detected by observing respective fluorescence.

H. pylori eradication rates of the patients with and without C resistance were compared statistically by Fischer's exact test. A p value less than 0.05 was accepted as statistically significant.

RESULTS

Forty-five patients enrolled in the study, and all were included for the intention to treat (ITT) analysis. Forty-three (95.6%) of them completed the protocol forming the basis for per protocol (PP) analysis. Two (4.4%) cases were lost to follow-up. There were 22 (51.2%) male and 21 (48.8%) female patients, and the average age was 46.3 ± 11.5 years. All of the 43 patients had used the drugs completely.

Duodenal ulcer (DU) was seen in 30.2% (n=13) and erythematous gastritis in 69.8% (n=30; antral gastritis in 14 and pangastritis in 16) of the patients on upper endoscopy. C resistance was studied in 26 patients. Ten (38.5%) patients were found to be C-resistant. Repeated upper endoscopy showed that ulcers of all 13 patients with DU were completely healed. *H. pylori* eradication was achieved in 35 patients (PP: 81.4%, ITT: 77.8%). *H. pylori* was eradicated in 13 (81.3%) of 16 C-sensitive patients and in 8 (80%) of 10 C-resistant patients (p>0.05) (Figure 1). None of the patients stopped the treatment due to adverse events. Mild side effects, i.e. taste perversion, emesis, and diarrhea, occurred in 16 (37.2%), 2 (4.6%), and 2 (4.6%) patients, respectively.

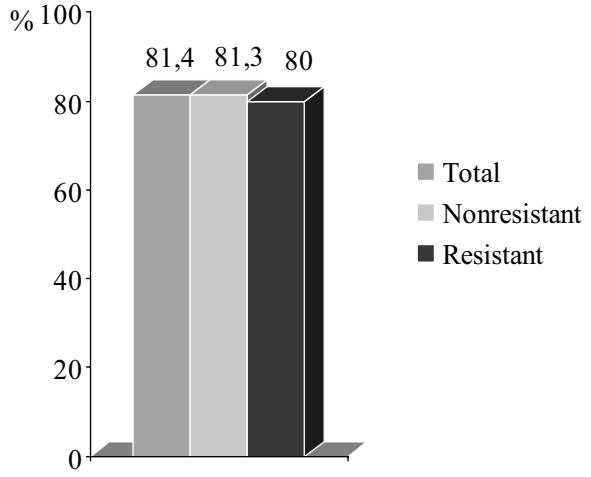

Figure 1. *H. pylor*i eradication rates of the patients according to clarithromycin resistance

DISCUSSION

Recommended treatments for *H. pylori* eradication are PPI- or RBC-based regimens with C and A or nitroimidazole (1). Triple regimens, particularly with PPI, have been widely used and reported as very successful. However, recent studies from many countries have begun to report the failure of these regimens (12, 13). *H. pylori* eradication therapy with a PPI plus A and C is the most popular treatment regimen in Turkey; however, success rates of *H. pylori* eradication with PPI-based regimens have been reported recently as being rather decreased in Turkey as well. A meta-analysis by Kadayıfçı et al. documented that average *H. pylori* eradication rate with PPI-based triple regimens was 84% in 1997, decreasing to 55.3% in 2004 (2). Similar results with one-week PPI-A-C triple regimen were also found in studies performed at the Gastroenterology Department of Ege University Medical School, with an eradication rate of 93.3% in 1996 and of 47.1% in 2004 (3, 14).

It is known that the most important factor affecting the success rate of *H. pylori* eradication therapy is resistance to antibiotics, especially to C. Success rates of *H. pylori* eradication therapy vary between 0% and 48% with PPI-C-A/or metronidazole in C-resistant patients (5). Resistance to metronidazole may not markedly affect the success rate of eradication. Graham et al. reported that *H. pylori* eradication rate of PPI-based regimens with nitroimidazole was 90% in metronidazole-sensitive patients, while it only decreased to 75% in metronidazole-resistant ones (15). Resistance to C has been reported as 8-30% and to metronidazole as 15-66% in the world (16). In Turkey, resistance to C was found to be 0% in 1998, with an impressive increase to 48.2% in 2004 (6, 7). Thus, recent national studies reporting the failure of *H. pylori* eradication therapies are not surprising when considering the impact of C resistance on the success rate of eradication.

On the other hand, in vitro studies suggest a synergistic activity between RBC and C. Osato et al. showed a synergy between RBC and C with a decrease of RBC minimal inhibitory concentrations (MICs) from more than 8 mg/L to less than 2 mg/L in 8 of 10 C-resistant *H. pylori* strains (9). RBC can release bismuth, which has also been shown to have moderate anti-*H. pylori* activity, in the gastric mucosa (17). Level of bismuth concentration achieved in the mucosa is very important and markedly higher than the MIC. PPIs have anti-H.pylori activity at high concentrations, which are unlikely to be achieved in vivo (18). When used together with a PPI, anti-*H. pylori* activity of C is mostly due to an elevated pH, which decreases the MIC of C, and possibly to decreased volume of secretion, which may in turn increase the C concentration (19, 20). Furthermore, in vivo studies support this condition. Megraud et al. reported eradication rates of 33% and 92% in C-resistant patients with omeprazole-C and RBC-C dual therapies, respectively (11). Houben et al. reported that in case of C resistance, a mean drop in efficacy of 56% was found for C-containing PPI-triple therapies, while in contrast, for RBC combined with C and nitroimidazole, no difference in efficacy was found in case of C resistance (5). In a study of

Bago et al., *H. pylori* eradication rates of C-resistant patients were found to be 40% and 80% with omeprazole-A-C and RBC-A-C therapies, respectively (10). In our study, treatment with RBC-A-C resulted in an eradication rate of 80% in C-resistant patients, which is similar to reported rates in the literature. In Turkey, other studies using RBC-A-C triple regimen performed by Avşar et al. (21), Hatemi et al. (22), Alkım et al. (23), and Çınar et al. (24) documented *H. pylori* eradication rates as 74.6%, 76.7%, 87%, and 95.9%, respectively. The study by Çınar et al. is especially remarkable since the reported eradication rate is the highest one among the studies published recently in Turkey. However, C resistance was investigated in none of these studies.

In another study performed at the Gastroenterology Department of Ege University Medical School, we found that *H. pylori* eradication rates in C-resistant patients were 26.7% and 60% with one- and two-week PPI-A-C triple regimens, respectively (3). These results were consistent with the results of the studies in the literature which mention the low success rates of eradication therapies with PPI-based regimens in C-resistant patients.

In conclusion, we have found that two-week therapy with RBC-A-C is very effective for *H. pylori* eradication in C-resistant patients. We suggest that RBC-A-C combination should be used as a first-line eradication therapy regimen since C resistance has been progressively increasing in Turkey.

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