PO Box 2345, Beijing 100023, China
www.wjgnet.com
wjg@wjgnet.com

ELSEVIER

World J Gastroenterol 2005;11(6):842-845
World Journal of Gastroenterology ISSN 1007-9327

• *BRIEF REPORTS* •

Helicobacter pylori infection in hemodialysis patients: Susceptibility to amoxicillin and clarithromycin

Selim Aydemir, Sedat Boyacioglu, Gurden Gur, Muge Demirbilek, Fusun Kamber Can, Murat Korkmaz, Ugur Yilmaz

Selim Aydemir, Department of Gastroenterology, Zonguldak Karaelmas University Faculty of Medicine, 67800, Zonguldak, Turkey
Sedat Boyacioglu, Gurden Gur, Murat Korkmaz, Ugur Yilmaz, Department of Gastroenterology, Baskent University Faculty of Medicine, 06000, Ankara, Turkey
Muge Demirbilek, Fusun Kamber Can, Department of Clinical Microbiology, Baskent University Faculty of Medicine, 06000, Ankara, Turkey
Correspondence to: Dr. Selim Aydemir, Department of Gastroenterology, Zonguldak Karaelmas University Faculty of Medicine, 67800, Zonguldak, Turkey. selimaydemir@hotmail.com
Telephone: +90-372-2576169
Received: 2004-07-05 Accepted: 2004-10-13

Abstract

AIM: To evaluate susceptibility of *Helicobacter pylori* to amoxicillin and clarithromycin in end-stage renal disease (ESRD) patients and non-uremic controls.

METHODS: The subjects with dyspeptic complaints were 33 ESRD patients and 46 age- and sex-matched non-uremic controls who exhibited *H pylori* on antral biopsy specimens. The two groups were age and sex matched. The *H pylori* strains' pattern of susceptibility to amoxicillin and clarithromycin was investigated with the agar dilution technique.

RESULTS: None of the *H pylori* strains from either group showed resistance to amoxicillin with the agar dilution method. Twelve (36.4%) of the ESRD group strains and 7 (15.2%) of the control group strains showed resistance to clarithromycin, and this difference was statistically significant ($P<0.05$).

CONCLUSION: Resistance to amoxicillin does not appear to be an important problem in *H pylori*-infected ESRD and non-uremic patients in our region. In contrast, the rates of resistance to clarithromycin are high, particularly in the ESRD population.

Key words: *Helicobacter pylori*; Chronic renal failure; Antibiotic resistance; Clarithromycin; Amoxicillin

Aydemir S, Boyacioglu S, Gur G, Demirbilek M, Can FK, Korkmaz M, Yilmaz U. *Helicobacter pylori* infection in hemodialysis patients: Susceptibility to amoxicillin and clarithromycin. *World J Gastroenterol* 2005; 11(6): 842-845
http://www.wjgnet.com/1007-9327/11/842.asp

INTRODUCTION

Helicobacter pylori (*H pylori*) infection is the most common chronic bacterial infection in humans. Estimates indicate that approximately 60% of the world population is colonized with this agent. In addition to the direct effects of *H pylori*, complications related to infection with this bacterium are serious public health problems[1]. *H pylori* is a gram-negative, spiral-shaped bacillus that colonizes the antrum of the stomach causing type B chronic-active antral gastritis. Research has established that a number of diseases of the gastrointestinal system are linked with *H pylori* infection. Specifically, this condition is known to play an important role in the pathogenesis of peptic ulcer disease, and is also associated with gastric cancer[2,3].

The ideal treatment for *H pylori* infection is yet to be discovered. Triple-agent protocols involving a proton-pump inhibitor and two antibiotics are frequently used[4-6]. Most patients with *H pylori* infection respond well to these regimens; however, therapy may fail due to antibiotic resistance, poor compliance, or treatment-related factors (number/doses of combined medications, dosing frequency, and/or treatment duration). Antibiotic resistance is an important cause of *H pylori* treatment failure. In order to administer optimal treatment in a given region, it is critical to know the antibiotic susceptibility pattern for the strains that exist in the area[7-10].

The epidemiological data concerning *H pylori* infection in end-stage renal disease (ESRD) patients are insufficient[11]. Most studies of the epidemiological features of *H pylori* infection have revealed similar findings in ESRD and non-uremic patients[11]. The prevalence rates of *H pylori* infection and dyspeptic complaints in ESRD patients are high. Most individuals with ESRD exhibit immune system dysfunction, and these patients also tend to require frequent invasive procedures. These factors result in increased rates of infection, and this explains the very high frequencies of antibiotic use in this population.

We designed a controlled study to investigate possible differences in antibiotic resistance pattern for *H pylori*-infected ESRD patients and non-uremic controls at a major center in Ankara, Turkey.

MATERIALS AND METHODS

Subjects

Thirty-three ESRD patients who arrived at our gastroenterology clinic with dyspeptic complaints were enrolled in the study. Forty-six dyspeptic control patients with normal renal function and no other known health problems also participated. The ESRD group comprised of 17 (51.5%)

females and 16 (48.5%) males. The mean age of these patients was 42±14 years, and the mean hemodialysis duration 4.1±2.8 years. The control group comprised of 26 (56.5%) females and 20 (43.5%) males of mean age 43±14 years (Table 1). The two groups were age and sex matched (P>0.05). Each of the 79 subjects was investigated with upper gastrointestinal endoscopy. The exclusion criteria were as follows: prior treatment for *H pylori* infection; use of any antibiotic, proton-pump inhibitor, H_2-receptor antagonist or bismuth-containing drugs in the 30 d before the study; negative *H pylori* culture from an antral biopsy specimen; and positive *H pylori* culture but insufficient growth for antibiotic susceptibility testing.

The study was conducted according to the guidelines of the Declaration of Helsinki. The study protocol was approved by the Local Ethics Committee of Baskent University. Patients were enrolled in the study after getting their written informed consent.

Endoscopic procedure

All the upper gastrointestinal system endoscopy procedures were performed under appropriate sedation (lidocaine 10 mg/puff for pharyngeal anesthesia and intravenous midazolam 2.5-7.5 mg for premedication) using the same videoendoscope (Olympus GIF Q240). Two biopsy specimens were collected in each case, one from the pre-pyloric region and one from the incisura angularis. Tissues were placed in selective medium (Brucella broth culture containing 20% glycerol) for transfer to the laboratory.

Culture

Within 4 h of the endoscopy procedure, each biopsy specimen was placed in 0.5 mL sterile saline and homogenized in a tissue homogenizer. Two droplets of each homogenate were placed in 7% sheep blood Brucella agar, and the plates were incubated in microaerophilic conditions at 37 ℃ for 3-7 d. Another sample was gram stained and examined under the microscope for gram-negative spiral bacilli. Isolates from the cultures were identified as *H pylori* based on typical colony morphology on selective agar, and with gram staining and oxidase, catalase and urease tests.

The bacteria cultured from each case were kept in separate cryo vials with 0.75 mL of 20% glycerol containing Brucella broth medium at -80 ℃ until susceptibility testing was done.

Minimum inhibitor concentration study

Each stock cultured specimen was warmed, re-plated on 7% sheep blood Brucella agar medium, and incubated in microaerophilic conditions at 37 ℃ for 3-5 d. The pure cultured *H pylori* strains were then tested for susceptibility to amoxicillin and clarithromycin using the agar dilution method, in accordance with the National Committee for Clinical Laboratory Standards (NCCLS)[12-14]. Using appropriate solvents, two-fold serial dilutions of each antibiotic were prepared resulting in final concentration ranges of 0.015-4.0 μg/mL for amoxicillin (Sigma Chemical Co.) and 0.015-8.0 μg/mL for clarithromycin (Abbott Laboratories). Five percent sheep blood containing Mueller-Hinton agar was used for the agar dilution method. Multiple suspensions of each *H pylori* strain to be tested were prepared in sterile saline, and were arranged in order of clarity according to the MacFarland 2 standard technique. Then a 3-μL droplet of each suspension was placed in each of the above-described antibiotic dilutions and on control plates. All strains were studied in pairs, and the *H pylori* strain NCTC 11637 was used as a control. The inoculated dilution tubes and plates were incubated in microaerophilic conditions at 37 ℃ for 3 d. For each isolate, the dilution tube with the lowest antibiotic concentration that showed no visible growth was recorded as the minimum inhibitory concentration (MIC). To determine whether or not a strain was susceptible to each antibiotic, we compared the recorded MIC values for amoxicillin and clarithromycin to previously reported MIC cut-off values for these drugs (0.5 and 1 μg/mL, respectively)[14-17].

Statistical analysis

Numeric values were expressed as mean±SD. The χ^2 test was used to compare mean values, and the Mann-Whitney U test was used to compare numeric values. P values <0.05 were considered to indicate statistical significance.

RESULTS

A total of 79 *H pylori* strains (one from each ESRD patient and control subject) were isolated from the antral biopsy specimens. The demographics for the two study groups are shown in Table 1. None of the *H pylori* strains from either group showed resistance to amoxicillin. Twelve (36.4%) of the 33 ESRD group strains and 7 (15.2%) of the 46 control group strains showed resistance to clarithromycin, and this difference was statistically significant (P = 0.03) (Table 1).

Table 2 shows the demographic characteristics of the ESRD patients and control subjects with clarithromycin-resistant and clarithromycin-susceptible *H pylori* strains. Of

Table 1 Demographic characteristics and frequencies of antibiotic resistance in the two study groups

	Non-uremic patients n = 46	ESRD patients n = 33	P
Mean age (yr)	43±14	42±14	>0.05
Female/Male	26/20 (43.5%)	17/16 (48.5%)	>0.05
Hemodialysis duration (yr)		4.1±2.8	
Clarithromycin resistance	15.2% (7/46)	36.4% (12/33)	<0.05
Amoxicillin resistance	0% (0/46)	0% (0/33)	

Table 2 Demographic characteristics of the ESRD and non-uremic patients with clarithromycin-resistant and susceptible *H pylori* strains

	Resistant	Susceptible	P
ESRD patients			
n	12	21	
Mean age (yr)	42.9 ±12.6	40.9 ±14.7	>0.05
Female/Male	7/5	10/11	
Hemodialysis duration (yr)	5.6 ±3.8	3.3 ±1.8	>0.05
Non-uremic patients			
n	7	39	
Mean age (yr)	49 ±17	42 ±14	>0.05
Female/Male	4/3	22/17	

the twelve ESRD patients with *H pylori* strains that were resistant to clarithromycin, five were males and seven were females. The mean age of these patients was 42.9±12.6 years, and the mean age in the susceptible cases was 40.9±14.7 years. This age difference was not statistically significant ($P>0.05$). The mean hemodialysis durations in the subgroups of ESRD patients with clarithromycin-resistant and susceptible *H pylori* strains were 5.6±3.8 years and 3.3±1.8 years, respectively ($P>0.05$).

Of the seven non-uremic control patients with *H pylori* strains that were resistant to clarithromycin, three were males and four were females. The mean age of these patients was 49±17 years, and the mean age in the susceptible cases was 42±14 years. This age difference was not statistically significant ($P>0.05$).

DISCUSSION

H pylori infection is the most widespread bacterial infection in the world. Antibiotic resistance is important in the management of these patients, and knowledge of resistance pattern helps the clinician to establish the most appropriate treatment strategy[18-20].

The therapy for *H pylori* infection in ESRD patients and non-uremic patients are approximately the same[21]. Drugs that contain bismuth may be toxic for individuals with ESRD, and are generally avoided in this population[11]. The combination of proton-pump inhibitors, clarithromycin and amoxicillin is frequently used to treat *H pylori* infection in both uremic and non-uremic patients[4,20,22].

For some antibiotics, the *in vivo* and *in vitro* findings for *H pylori* susceptibility differ. Rates of antibiotic resistance in *H pylori* infection, particularly resistance to clarithromycin, are rising dramatically in Turkey and many other countries throughout the world, so it is more important than ever to be aware of this issue[23,24].

One important barrier in the approach to this problem is the lack of a standard system for assessing *H pylori* microorganism susceptibility to antibiotics. The NCCLS subcommittee recommends the agar dilution method for this purpose, and this was our basis for using this technique in our study[14]. The NCCLS MIC value for *H pylori* resistance to clarithromycin is 1 μg/mL. There is no standard MIC value for *H pylori* resistance to amoxicillin, so we used the lowest value in the literature (0.5 μg/mL) as the cut-off in our study[14-17].

The *in vitro* efficacy of amoxicillin for *H pylori* infection is high; however, when amoxicillin is used as a single therapeutic agent, this rate drops to approximately 20% due to low efficacy at acid pH[25]. This is the reason why amoxicillin is used in combination with anti-secretory drugs[23]. A recent report has identified a number of *H pylori* strains that are resistant to amoxicillin[26-29], but none of the isolates in our study showed resistance to this agent.

Clarithromycin is the antibiotic to which *H pylori* is most sensitive *in vitro*, and this agent is widely used in combination regimens. Oral administration yields high serum and tissue concentrations, and the drug is stable in acid environments. Some authors have identified resistance to clarithromycin as the most important problem in *H pylori* treatment failure[30,31]. It has been reported that clarithromycin resistance results in significantly lower *H pylori* eradication rates[7], and studies from various nations have documented increasing rates of *H pylori* resistance to this agent in recent years[32-34]. Rates of *H pylori* resistance to clarithromycin are higher in countries where macrolide antibiotics are frequently used[32]. The estimated range of resistance to this antibiotic in *H pylori*-infected patients in various nations and regions of the world is 1-15%[32,35,36].

In our *H pylori*-infected study subjects, the rates of *H pylori* resistance to clarithromycin were 15.2% in the otherwise healthy controls and 36.4% in the ESRD patients. These figures are both very high, and provide further evidence that *H pylori* resistance to clarithromycin is on the rise in our country. If this problem continues to escalate, there will be even more serious problems treating *H pylori* infection in future. In addition to the high frequencies we observed in both study groups, the significantly higher rate of clarithromycin resistance in our ESRD group compared to the controls is of particular concern. It is important to be aware that the risk of *H pylori* resistance to this agent is likely to be higher in a patient who takes antibiotics for a variety of reasons. It is essential to know the antibiotic susceptibility of *H pylori* strains in order to identify what drug combination will be effective and how to prevent antibiotic resistance, to avoid using inappropriate drugs, and to decrease costs. To our knowledge, this is the first study that has examined *H pylori* resistance to amoxicillin and clarithromycin in *H pylori*-infected ESRD patients.

In conclusion, it appears that *H pylori* resistance to amoxicillin is not a major problem in ESRD and non-uremic patients in Turkey. However, the *H pylori* isolates from the ESRD patients in this series showed high rates of clarithromycin resistance, and expansion of this problem in all patient groups is a concern worldwide. Patients with ESRD have a high probability of *H pylori* resistance to clarithromycin due to immune compromise and extensive use of antibiotics. In order to effectively treat *H pylori* in this patient group, it is important to know the antibiotic susceptibility pattern of the *H pylori* microorganism present in each individual.

REFERENCES

1 **Cave DR**. Transmission and epidemiology of *Helicobacter pylori*. *Am J Med* 1996; **100**: 12S-17S; discussion 17S-18S
2 **Misiewicz JJ**. Current insights in the pathogenesis of *Helicobacter pylori* infection. *Eur J Gastroenterol Hepatol* 1995; **7**: 701-703
3 **Wyle FA**. *Helicobacter pylori*: current perspectives. *J Clin Gastroenterol* 1991; **13** Suppl 1: S114-S124
4 **Malfertheiner P**, Megraud F, O'Morain C, Hungin AP, Jones R, Axon A, Graham DY, Tytgat G. Current concepts in the management of *Helicobacter pylori* infection-the Maastricht 2-2000 Consensus Report. *Aliment Pharmacol Ther* 2002; **16**: 167-180
5 **Misiewicz JJ**. Management of *Helicobacter pylori*-related disorders. *Eur J Gastroenterol Hepatol* 1997; **9** Suppl 1: S17-S20; discussion S20-S21, S27-S29
6 **van der Hulst RW**, Keller JJ, Rauws EA, Tytgat GN. Treatment of *Helicobacter pylori* infection: a review of the world literature. *Helicobacter* 1996; **1**: 6-19
7 **Tompkins DS**, Perkin J, Smith C. Failed treatment of *Helicobacter pylori* infection associated with resistance to clarithromycin. *Helicobacter* 1997; **2**: 185-187

8 **Megraud F**. *H pylori* antibiotic resistance: prevalence, importance, and advances in testing. *Gut* 2004; **53**: 1374-1384

9 **Perri F**, Qasim A, Marras L, O'Morain C. Treatment of *Helicobacter pylori* infection. *Helicobacter* 2003; **8** Suppl 1: 53-60

10 **McMahon BJ**, Hennessy TW, Bensler JM, Bruden DL, Parkinson AJ, Morris JM, Reasonover AL, Hurlburt DA, Bruce MG, Sacco F, Butler JC. The relationship among previous antimicrobial use, antimicrobial resistance, and treatment outcomes for *Helicobacter pylori* infections. *Ann Intern Med* 2003; **139**: 463-469

11 **Fabrizi F**, Martin P. *Helicobacter pylori* infection in patients with end-stage renal disease. *Int J Artif Organs* 2000; **23**: 157-164

12 **Midolo PD**, Bell JM, Lambert JR, Turnidge JD, Grayson ML. Antimicrobial resistance testing of *Helicobacter pylori*: a comparison of Etest and disk diffusion methods. *Pathology* 1997; **29**: 411-414

13 **Osato MS**. Antimicrobial susceptibility testing for *Helicobacter pylori*: sensitivity test results and their clinical relevance. *Curr Pharm Des* 2000; **6**: 1545-1555

14 **Osato MS**, Reddy R, Reddy SG, Penland RL, Graham DY. Comparison of the Etest and the NCCLS-approved agar dilution method to detect metronidazole and clarithromycin resistant *Helicobacter pylori*. *Int J Antimicrob Agents* 2001; **17**: 39-44

15 **Adamek RJ**, Suerbaum S, Pfaffenbach B, Opferkuch W. Primary and acquired *Helicobacter pylori* resistance to clarithromycin, metronidazole, and amoxicillin-influence on treatment outcome. *Am J Gastroenterol* 1998; **93**: 386-389

16 **Kalach N**, Bergeret M, Benhamou PH, Dupont C, Raymond J. High levels of resistance to metronidazole and clarithromycin in *Helicobacter pylori* strains in children. *J Clin Microbiol* 2001; **39**: 394-397

17 **Torres J**, Camorlinga-Ponce M, Perez-Perez G, Madrazo-De la Garza A, Dehesa M, Gonzalez-Valencia G, Munoz O. Increasing multidrug resistance in *Helicobacter pylori* strains isolated from children and adults in Mexico. *J Clin Microbiol* 2001; **39**: 2677-2680

18 **Megraud F**. *Helicobacter pylori* resistance to antibiotics: prevalence, mechanism, detection. What's new? *Can J Gastroenterol* 2003; **17** Suppl B: 49B-52B

19 **Megraud F**. Basis for the management of drug-resistant *Helicobacter pylori* infection. *Drugs* 2004; **64**: 1893-1904

20 **Parsonnet J**. *Helicobacter pylori*. *Infect Dis Clin North Am* 1998; **12**: 185-197

21 **Munoz de Bustillo E**, Sanchez Tomero JA, Sanz JC, Moreno JA, Jimenez I, Lopez-Brea M, Pajares JM, Traver JA. Eradication and follow-up of *Helicobacter pylori* infection in hemodialysis patients. *Nephron* 1998; **79**: 55-60

22 **Bazzoli F**, Bianchi Porro G, Bianchi MG, Molteni M, Pazzato P, Zagari RM. Treatment of *Helicobacter pylori* infection. Indications and regimens: an update. *Dig Liver Dis* 2002; **34**: 70-83

23 **Harris AW**, Misiewicz JJ. Eradication of *Helicobacter pylori*. *Baillieres Clin Gastroenterol* 1995; **9**: 583-613

24 **Pamuk ON**, Pamuk GE, Celik A, Uzunismail H. Are Turkish *Helicobacter pylori* strains gaining resistance against clarithromycin? *Am J Gastroenterol* 2000; **95**: 1839-1840

25 **Walter LP**, David YG. *Helicobacter pylori*. In: Feldman M, Friedman LS, Sleisenger MH eds. Gastrointestinal and liver disease. 6 ed. *Philadelphia: WB Saunders* 1998: 604-619

26 **van Zwet AA**, Vandenbroucke-Grauls CM, Thijs JC, van der Wouden EJ, Gerrits MM, Kusters JG. Stable amoxicillin resistance in *Helicobacter pylori*. *Lancet* 1998; **352**: 1595

27 **Glupczynski Y**, Megraud F, Lopez-Brea M, Andersen LP. European multicentre survey of *in vitro* antimicrobial resistance in *Helicobacter pylori*. *Eur J Clin Microbiol Infect Dis* 2001; **20**: 820-823

28 **Megraud F**. Resistance of *Helicobacter pylori* to antibiotics and its impact on treatment options. *Drug Resist Updat* 2001; **4**: 178-186

29 **Boyanova L**, Mentis A, Gubina M, Rozynek E, Gosciniak G, Kalenic S, Goral V, Kupcinskas L, Kantarceken B, Aydin A, Archimandritis A, Dzierzanowska D, Vcev A, Ivanova K, Marina M, Mitov I, Petrov P, Ozden A, Popova M. The status of antimicrobial resistance of *Helicobacter pylori* in eastern Europe. *Clin Microbiol Infect* 2002; **8**: 388-396

30 **Lind T**, Megraud F, Unge P, Bayerdorffer E, O'Morain C, Spiller R, Veldhuyzen Van Zanten S, Bardhan KD, Hellblom M, Wrangstadh M, Zeijlon L, Cederberg C. The MACH2 study: role of omeprazole in eradication of *Helicobacter pylori* with 1-week triple therapies. *Gastroenterology* 1999; **116**: 248-253

31 **Logan RP**, Gummett PA, Schaufelberger HD, Greaves RR, Mendelson GM, Walker MM, Thomas PH, Baron JH, Misiewicz JJ. Eradication of *Helicobacter pylori* with clarithromycin and omeprazole. *Gut* 1994; **35**: 323-326

32 **Alarcon T**, Domingo D, Lopez-Brea M. Antibiotic resistance problems with *Helicobacter pylori*. *Int J Antimicrob Agents* 1999; **12**: 19-26

33 **Ellenrieder V**, Boeck W, Richter C, Marre R, Adler G, Glasbrenner B. Prevalence of resistance to clarithromycin and its clinical impact on the efficacy of *Helicobacter pylori* eradication. *Scand J Gastroenterol* 1999; **34**: 750-756

34 **Xia HX**, Fan XG, Talley NJ. Clarithromycin resistance in *Helicobacter pylori* and its clinical relevance. *World J Gastroenterol* 1999; **5**: 263-266

35 **Ferrero M**, Ducons JA, Sicilia B, Santolaria S, Sierra E, Gomollon F. Factors affecting the variation in antibiotic resistance of *Helicobacter pylori* over a 3-year period. *Int J Antimicrob Agents* 2000; **16**: 245-248

36 **Street ME**, Caruana P, Caffarelli C, Magliani W, Manfredi M, Fornaroli F, de'Angelis GL. Antibiotic resistance and antibiotic sensitivity based treatment in *Helicobacter pylori* infection: advantages and outcome. *Arch Dis Child* 2001; **84**: 419-422

Edited by Gabbe M