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Identification of the Prevalence of Resistance to Clarithromycin in Helicobacter Pylori Isolated from Gastric Biopsy via PCR Method

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ABSTRACT

BACKGROUND AND OBJECTIVE: Helicobacter pylori infection is the most common worldwide human infection, affecting approximately half of the world's population. One of the most important causes of failure in the treatment of helicobacter pylori infection is its resistance to antibiotics. The purpose of this study was to evaluate the antibiotic resistance of helicobacter pylori, isolated from patients, referring to Shahid Beheshti Hospital of Shiraz, Iran.

METHODS: In this cross-sectional study, biopsy samples were obtained from 80 patients, referring to the endoscopy department of Shahid Beheshti Hospital of Shiraz. Biopsy samples were transferred to the laboratory in thioglycollate broth. After conducting biochemical tests for final confirmation, PCR tests were performed. In order to perform an antibiotic resistance test, Clinical and Laboratory Standards Institute (CLSI) method was applied.

FINDINGS: Out of 80 cases, 59 samples (73.75%) were infected with helicobacter pylori. Among these cases, 36 samples (61%) were resistant to metronidazole, 19 samples (32.2%) were resistant to amoxicillin, and 14 samples (23.72%) were resistant to tetracycline. As the results indicated, resistance to clarithromycin was the lowest, reported in two cases (3.35%).

CONCLUSION: Considering the high resistance of samples to metronidazole, it is necessary to replace this antibiotic with clarithromycin or other antibiotics in therapeutic regimens.

KEY WORDS: Helicobacter Pylori, Antibiotic Resistance, Clarithromycin.

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Introduction

Helicobacter pylori (abbreviated as H. pylori) infection has a high global prevalence, occurring at all age ranges. The prevalence of infections with H. pylori varies in different world regions and is highly dependent on the general living condition in each region (1, 2). H. pylori has been shown to exhibit circular and creeping movements, which are faster in viscous environments such as gastric mucosa (1, 3). A large number of these bacteria may be mostly found on the gastric mucosal surface of human carriers and can survive by penetrating into mucin sub-layers.

Despite the acquired immune and inflammatory responses and the continuous replacement of gastric epithelial cells, H. pylori is able to infect the gastric mucosa for decades during one's life (4). H. pylori is a significant factor for gastric diseases and peptic ulcers and is considered a risk factor for gastric cancer and B-cell lymphoma of mucosa-associated lymphoid tissue (MALT). After isolating and culturing H. pylori from the gastric biopsies of patients with gastritis in 1983 and introducing it as the major factor for chronic active gastritis, researchers' attention was drawn to this bacterium. Many studies are annually conducted on this subject, and the relationship between this bacterium and various diseases of the digestive system such as non-ulcer dyspepsia (NUD), peptic ulcer, duodenal ulcer, stomach cancer, and gastric lymphoma has been demonstrated (1,5,6). Infections with H. pylori are highly common. In some societies, 69% of people, aged 16-69 years, and 79.2% of people within the age range of 46-55 years are infected with this bacterium. Even though the exact transmission route of H. pylori is unclear, this type of infection can be caused by consuming unwashed or uncooked vegetables grown in contaminated soil with human excrement (in developing countries), polluted water, or even dental or mouth plaques (7, 8). The treatment of H. pylori infection includes a combination of three or four medicines including amoxicillin, clarithromycin, tetracycline, metronidazole, bismuth compounds, and a proton pump inhibitor. Use of different types of antibiotics can improve disease elimination and decrease the risk of resistance to antibiotics. The dosage of antibiotics used for the treatment of H. pylori infection is higher than the dose used for other infections; this matter is of high significance for achieving higher drug concentrations in gastric mucosa. A combination of proton pump inhibitors and antibiotics can prevent the decomposition of antibiotics in an acidic gastric luminal pH. Since proton pump inhibitors and H2 blockers are not able to adjust pH to 7, antibiotics, which are used for the treatment of H. pylori infections, should be at least functional in acidic environments (9). In most studies conducted in Iran, resistance to metronidazole has been reported at 60-70% (10). In a study by Siavashi et al. in Tehran, Fallahi et al. at Tehran University, Savari et al. in Kerman, and Rafeey et al. at Tabriz University, the resistance of isolated H. pylori to metronidazole was reported to be 95%, 35%, 71%, and 54.16%, respectively (11-14). Moreover, Kohanteb et al. and Tomatari et al. reported the rate of resistance to metronidazole in isolated H. pylori of patients with dyspepsia to be 72.6% and 64%, respectively (15, 16). Additionally, resistance to clarithromycin ranges between 3% and 50%, worldwide (17, 18). On the other hand, resistance to amoxicillin has been reported to be around 0% (17, 19). Considering the difficulties in culturing samples and performing standard tests for determining sensitivity to H. pylori, there is little information at hand about the antibiotic resistance of these bacteria in Fars province and Iran in general. Therefore, the purpose of this study was to investigate the resistance of H. pylori, obtained from the gastric biopsies of patients referring to Shahid Beheshti Hospital in Shiraz, to common antibiotics in three- and four-drug treatments.

Methods

This cross-sectional study was conducted on 80 patients, selected via simple sampling, referring to the endoscopy department of Shahid Beheshti Hospital of Shiraz in the spring of 2014. Biopsies of the gastric antrum were performed using endoscopy and the results were reported by a gastroenterologist. The required materials and media were transferred to the endoscopy department. The samples were transferred to the department of microbiology at Islamic Azad University of Kazeroon, using brain-heart infusion medium. The biopsy samples were cultured on a Colombia agar medium, enriched with 5% defibrinated sheep blood, 7% fetal calf serum (FCS), vancomycin, polymyxin, and trimethoprim under microaerophilic conditions at 37°C for 5-6 days (20). Colonies suspected for H. pylori were evaluated, using biochemical, oxidase, catalase, and urease tests.

Polymerase chain reaction (PCR): DNA extraction of the collected samples was performed using a ready-

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to-use commercial kit (DBeasy tissue mini-kit, Hilden, Germany). The extracted DNAs were immediately transferred to an environment at -20 °C. Molecular analysis was performed at a final volume of 20 µl, containing 100 ng of the extracted DNA, 2 µl of X10 buffer, 0.2 µM of dNTP, 1.5 µM of $MgCl_2$, 25 pmol/uL of the primer, and 0.5 unit of Taq DNA polymerase. The sequence of primers is shown in table 1(21). The PCR product was finally placed on electrophoresis gel and the final result was investigated under ultraviolet light.

Drug resistance pattern: Disk diffusion method was applied to determine the sensitivity of the isolated bacterial H. pylori to antibiotic compounds. First, the colonies were reached to 0.5 McFarland in Mueller-Hinton broth. Then, the culture was prepared on Mueller-Hinton broth, enriched with 7% defibrinated sheep blood. Simultaneously, the disk containing antibiotics (purchased from Padtan Medicine Company, Iran) was placed on the culture medium. After 5 days of incubation at 37°C under microaerophilic conditions, the inhibition zone diameter of H. pylori was determined. Four antibiotic disks including metronidazole (5gµ), tetracycline (30 gµ), amoxicillin (25 gµ), and clarithromycin (15 gµ) were used via Kirby and Bauer standard method (22, 23).

Result

In this study, the biopsy results of 40 men and 40 women were evaluated. Among 59 subjects (73.75%) with positive culture results, 28 were male and 21 were female (fig 1). Overall, the mean age of subjects with H. pylori infection was 39 years. Among the infected subjects, 14 cases had NUD, 20 cases had duodenal ulcers, 24 cases had gastric ulcers, and only one subject had gastric cancer.

Among the infected samples, 36 cases (61%) were resistant to metronidazole, 19 cases (32.2%) were resistant to amoxicillin, and 14 cases (23.72%) were resistant to tetracycline As the results indicated, resistance to clarithromycin was the lowest, reported in two cases (3.35%) (table 2).

Table 1. Primers used for the identification of ureC gene

Primers	Sequence (3´-5´)	Product size (Bp)
UreC gene	F: GGA TAA GCT TTT AGG GGT GTT AGG GG R: GCT TAC TTT CTA ACA CTA ACG CGC	294

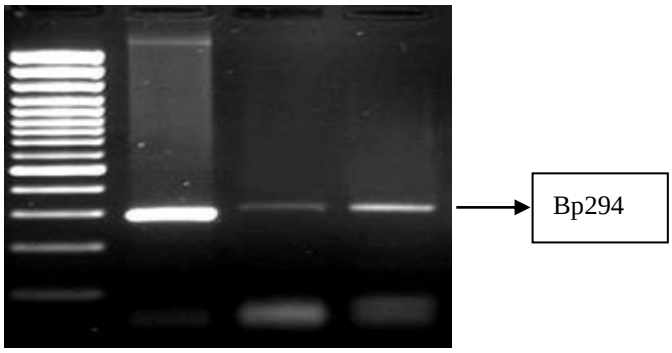

Figure 1. The results of PCR samples on 1.5% Agarose gel; column 1 on the left side: 100 bp marker, column 2 on the left side: positive control (ATCC 26695 H. pylori), columns 3 and 4 on the left side: H. pylori samples

Table 2. The prevalence of isolated H. pylori according to clinical samples

Results / Type of the disease	Positive ureC cases N(%)	Negative ureC cases N(%)	Total N(%)
Stomach cancer	1 (1.69)	1 (4.76)	2 (2.5)
Duodenal ulcer	20 (33.89)	7 (33.33)	27 (33.75)
Gastric ulcer	24 (40.67)	10 (47.61)	34 (42.5)
NUD	14 (23.72)	3 (14.28)	17 (21.25)
Total	59(100)	21(100)	80(100)

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Discussion

The results of this study showed that 73.75% of cases (28 men and 21 women) were infected with H. pylori. In countries such as Japan, South America, Turkey, and Pakistan, the prevalence of H. pylori infections is higher than 80%. However, in the UK and Nordic countries, the prevalence of these infections is reported to be between 20% and 40% (24, 25); the current findings were in consistence with the mentioned reports. The mean age of patients with H. pylori infection was 39 years in our study. Research performed in Iran showed the prevalence of this infection (in patients aged 35-55 years) to be 88.4-93%. Also, in patients aged 6-20 years in Ardebil and Yazd provinces, the prevalence of H. pylori infection was estimated at 47.5% and 30.6%, respectively. In patients aged 10-25 years in Tehran, the prevalence of this infection was estimated at 44.9% (26, 27), which is consistent with the current results.

Many studies have been carried out regarding the resistance of H. pylori strains to all types of antibiotics. The majority of these studies have suggested that H. pylori strains are not resistant to amoxicillin. Studies performed in Argentina (28), Japan (29), Bulgaria (30), Mexico (31), France (32), Italy (33), and New Zealand (34) showed the resistance rate to be zero, whereas this rate was estimated at 0.4% in Saudi Arabia (22), 29% in Brazil (35), and 71.9% in China (23).

In the current study, the resistance rate was reported to be 32.2%, which is obviously higher than that reported in most similar studies. Additionally, among H. pylori isolates, 23.72% of samples were resistant to tetracycline. Also, a study by Mohammadi et al. in Iran showed the rate of resistance to amoxicillin to be 1.7%, which is much lower than that reported in our study (36). In our study, the highest rate of resistance was towards metronidazole, reported in 61% of H. pylori isolates.

Resistance to antibiotics varies in different countries. For instance, the resistance rate was estimated at 16% in Bulgaria (30), and 20-40% in Argentina (28), Japan (29), Mexico (31), Lebanon (37), USA (38), Portugal (39), Australia (40), Nigeria (41), and New Zealand (34). Moreover, this rate was estimated at 40-60% in Brazil (35), France (32), and Korea (42), and 60-80% in Saudi Arabia (22) and Germany (43). A study performed in Italy also showed the resistance rate to be 100% (33). The study by Mohammadi et al. in Iran showed the resistance rate to metronidazole to be 75.4% (36).

The results of the present study indicated that the lowest resistance rate of isolated H. pylori to clarithromycin was approximately 3.35%. The resistance of H. pylori to clarithromycin varies around the world. In countries including Portugal (29), South Korea (42), China (23), and Iran (33), the rate of resistance is 20% at its highest, whereas it is 58% in Germany (43) and 32% in Italy (33). In fact, clarithromycin in regions with resistance rates higher than 20% should be used with more caution (29).

The results of this study confirmed that the resistance of H. pylori to common antibiotics in medicinal regimens is increasing. On the other hand, we still suggest multi-drug regimens for the treatment of H. pylori infections until new antibiotics are stabilized. Finally, the importance of culturing and sensitivity determination for defining the drug resistance pattern of this bacterium in different geographical regions (before the onset of treatment) needs to be emphasized. It is suggested that clarithromycin be used along with other medicinal compounds in the studied region.

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