Antibiotic resistance of Helicobacter pylori in Mashhad, Iran

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Abstract

Objective: To evaluate Helicobacter pylori resistance to amoxicillin, clarithromycin, metronidazole and tetracycline in Mashhad, Iran.

Methods: The cross-sectional study was done from January to May 2008 in Mashhad, involving 185 patients who had been indicated for endoscopy and lesions had been found. Biopsy samples were assessed with histological evaluation, rapid urease test, and culture. Antibiotic resistance was assessed by the disc diffusion method. Data was analysed with SPSS 11.5 using chi-square and Fisher's exact test. P values of < 0.05 were regarded as statistically significant.

Results: Of the total patients, histological evaluations were positive in 124 (67%). Compared with histology, sensitivity and specificity of rapid urease test were 96.7% and 100%, respectively. In 82 (66.1%) patients with positive cultures, antibiotic resistance was found in 14 (17.1%) for clarithromycin; 53 (64.6%) for metronidazole; and 8 (9.8%) for amoxicillin. No resistance was observed for tetracycline. Moreover, 9 (64%) patients with resistance to clarithromycin had co-resistance to metronidazole.

Conclusion: Metronidazole is not recommended for treatment of Helicobacter pylori as a first-line drug. Also, considering the sensitivity and specificity of rapid urease test, we suggest this method as a suitable alternative for histology.

Keywords: Helicobacter pylori, Antibiotic resistance, Antibiotic. (JPMA 63: 336; 2013)

Introduction

Helicobacter pylori (H. pylori) has been reported as the cause of some frequent gastrointestinal disorders.[1,2] Gastroduodenal ulcer disease and gastric malignancies are caused by H. pylori.[2-6] This bacterium has been classified as a type-1 carcinogen in the World Health Organization report.[7] Infection with H. pylori is so common that more than 50% of the world's population is infected with this bacterium.[8] This bacterium is usually acquired in childhood.[2] Eradication of H. pylori has prophylactic effect on gastric malignancies.[5]

Several combined antibacterial regimens have been advised for the eradication of H. pylori. In European guidelines, clarithromycin and either amoxicillin or metronidazole are the first-line antibiotics.[3] Also, in other advised regimens tetracycline has been suggested.[5,9] Current anti-H. pylori therapies can fail in up to 30% of patients.[3,5] Antibiotic resistance is an essential cause of this failure.[3,10-12]

Over time, antibiotic resistance of H. pylori has increased.[13,14] Siavoshi et al reported that resistance to metronidazole and tetracycline increased in 2008

compared with that in 2005.[15] Also, Sun et al reported that in Shanghai the resistance to clarithromycin increased from 8.6% in 2000 to 20.7% in 2009.[16] Furthermore, Gao et al. declared that H. pylori resistance to metronidazole, clarithromycin and fluoroquinolone increased from 2000 to 2009 in China.[17]

The aim of this study was to evaluate H. pylori resistance to amoxicillin, clarithromycin, metronidazole and tetracycline in Mashhad city of Iran.

Patients and methods

The cross-sectional study was conducted from January to May 2008 at the Gastrointestinal Clinic of Ghaem Hospital, Mashhad, northeastern Iran. Patients with chronic dyspepsia, who had been indicated for endoscopy and had lesions in their endoscopy, were enrolled in the study. We did not intend to do endoscopy in patients with no indication for endoscopy, thus we selected these patients. Patients with a history of using 3 or 4 drugs for the treatment of H. Pylori, those with gastrointestinal malignancy, or those with recent (2 weeks before the evaluation) use of antibiotics, bismuth or proton pomp inhibitors (PPIs) were excluded because antibiotics and some other drugs can impair the results of sensitivity tests. Indications of endoscopy in the patients with chronic dyspepsia included incomplete treatment with antacid drugs, presence of suspicious signs (weight loss,

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anaemia of undetermined cause, gastrointestinal bleeding, dysphasia, icterus, lymphadenopathy, consistent vomiting and palpable abdominal mass), age over 45 years, history of heartburn for more than 5 years, and being suspicious of cancer or organic diseases. The patients were divided into 3 age groups: <30 years; 30-50 years; and >50 years. The study was approved by the Research and Ethical Committee of Mashhad University of Medical Sciences.

Uring consecutive sampling, the sample size was calculated by the equation:

$$n = \frac{z^2_{1-\frac{\alpha}{2}}pq}{d^2}$$

All the endoscopies were done by an Internal Medicine physician. The samples were taken from the antrum of the stomach. A part of each sample was specified for rapid urease test (RUT) and another part was specified for culture and gram staining.

For RUT, the samples were transferred to a sterile urease test broth environment. Phenol red indicator was then added. To assess the change of the samples colour, they were kept at 37°C and were observed for 1 hour. When the sample colour changed from yellow to pink, the result of the test was considered positive.

The biopsy samples were transferred to the laboratory for culture and staining in semi-solid (0.1% agar) normal saline. The samples were prepared for Gram staining and then examined through a microscope.

For culturing the samples, 7% foetal calf serum and 10% lysed horse blood and the antibiotics (vancomycin (10μg/L), amphotericin B (5μg/L) and trimethoprim (5μg/L)) were added to RUT media and Colombia agar base (Merck, Germany). The samples were then placed on it. It was incubated at 37°C for 3-5 days under microaerophilic conditions. H. Pylori was identified as gram-negative with spiral or curved rods, which produce urease, catalase and oxidase. Sensitivity and specificity of RUT and culture were compared with histology as the gold standard.

In cases with positive cultures, antibiotic resistance was assessed by using the disc diffusion method for amoxicillin, clarithromycin, metronidazole and tetracycline. Brain heart infusion broth (Merck, Germany) plates were used with added 10% foetal calf serum. Bacterial suspension (equivalent to Mac Farland no. 3 turbidity) was transferred on the surface of the plates.

After the surface of the plates got dried, the antibiotic discs were transferred to the surface of the plates. After 15 minutes, the plates were transferred into a microaerophilic condition and they were kept in this condition at 37° C. After 5 days, the inhibition zone diametre (IZD) was recorded. IZDs more than or equal to 14, 15, 17 and 20mm were considered to be susceptible to Amoxicillin, Metronidazole, Tetracycline and Clarithromycin, respectively.

Data was analysed with SPSS 11.5, using chi-square and Fisher's exact test. P values of <0.05 were regarded as statistically significant.

Results

A total of 185 individuals were enrolled in the study. Of them, 101 (54.6%) were female; histological evaluations were positive in 124 (67%); and 65 (52.4%) of them were female. There was no relationship between gender and H. pylori, infection.

The mean age of the patients was 41.3±15.2 years; ranging from 19 to 82 years.

Infection with H. pylori was more common in the older patients. There was a significant relationship between age and infection with H. pylori (p <0.012) (Table-1).

Compared with histology, sensitivity and specificity of RUT were 96.7% and 100%, and sensitivity and specificity of culture were 66.1% and 100%, respectively.

In 82 patients with positive cultures, antibiotic resistance was found in 14 (17.1%) patients for clarithromycin; 53 (64.6%) patients for metronidazole; and 8 (9.8%) for amoxicillin. No resistance was observed for tetracycline.

There was no relationship between age and resistance to clarithromycin, amoxicillin or tetracycline (p<0.05). However, resistance to metronidazole was more common in patients younger than 50 years of age (p<0.010) (Table-2).

There was no significant relationship between antibiotic resistance and gender (p<0.05).

In addition, resistance to 3 antibiotics (clarithromycin,

Table-1: Distribution of patients and positive histology in age groups.

Age	No. of patients	No. of patients with positive histology	Percentage of patientswith positive histology
<30 years old	64 (34.5%)	37	57.8%*
30-50 years old	84 (45.4%)	55	65.5%*
>50 years old	37 (20%)	32	86.5%*

* P-value<0.012

Table-2: The frequency of resistance to clarithromycin, metronidazole and amoxicillin in age groups.

Age	<30 years	30-50 years	>50 years	P-value*
Number of patients with positive cultures	25	38	19	
Resistance to clarithromycin	4 (16%)	7 (18%)	3 (16%)	0.955
Resistance to metronidazole	22 (88%)	22 (58%)	9 (47%)	0.010
Resistance to amoxicillin	2 (8%)	4 (11%)	2 (11%)	0.939

* Calculated using the chi-square test.

metronidazole and amoxicillin) was seen in 1 (1.2%) patient, 21 (25.6%) patients had no resistance to any of the 4 antibiotics; and 9 (64%) patients with resistance to clarithromycin had co-resistance to metronidazole.

Discussion

H. pylori resistance to antibiotics is a worldwide problem. Over time, H. pylori resistance to antibiotics is increasing.[13,14] Considering the role of this bacterium in some frequent gastrointestinal disorders (such as gastroduodenal ulcer disease) and gastric malignancies, determination of antibiotic resistance to H. pylori is an essential requirement.

A total of 185 individuals were enrolled in this study, and 67% of them were positive in terms of histological evaluations. In our study, there was no relationship between gender and infection. However, in Naja et al.'s study, infection with H. pylori was more common in males.[7]

In the present study, infection with H. pylori was more common in the older patients. This finding is consistent with earlier studies.[7,18]

Compared with histology, sensitivity and specificity of RUT were 96.7% and 100%, and sensitivity and specificity of culture were 66.1% and 100%, respectively. According to these findings, the use of RUT as an alternative method seems to be logical.

In our study, the antibiotic resistance was found to be 64.6% for metronidazole, which was in line with earlier findings. Resistance to metronidazole ranged from 31.5% in Ireland from 2007 to 2008[19] to 95.5% in South Africa in 2009.[11] In Iran, it was reported to be 73.4% in the north from 2007 to 2010,[20] 44% in the south between 2008 and 2009,[21] and 55.6% in the centre from 2005 to 2008.[15] In developing countries, it was reported to be 95.5% in South Africa in 2009,[11] and 56.8% in Tunisia from 2005 to 2007.[13] In developed countries, it was declared to be 50% in Shanghai (China) in 2009,[16] 31.5% in Ireland from 2007 to 2008,[19] and 61% in France between 2004 and 2007.[6]

In our study, the antibiotic resistance was found to be 17.1% for clarithromycin. In other studies, resistance to clarithromycin was between 7.3% in Iran from 2005 to 2008[15] and 44% in Iran between 2008 and 2009.[21] In Iran, it was reported to be 30% in the north from 2007 to 2010,[20] 44% in the south between 2008 and 2009[21] and 7.3% in the center from 2005 to 2008.[15] In developing countries, it was reported to be 14.6% in Tunisia from 2005 to 2007.[13] In developed countries, it was declared to be 20.7% in Shanghai (China) in 2009,[16] 13.2% in Ireland from 2007 to 2008[19] and 26% in France between 2004 and 2007.[6] Resistance to clarithromycin in our study was lower than other studies that were done in the north and the south of Iran, but higher than the study carried out in the centre of Iran. Also, it is similar to the studies that were done in Tunisia, Shanghai (China) and Ireland.

In our study, the antibiotic resistance was found to be 9.8% for amoxicillin. In other studies, resistance to amoxicillin ranged from no resistance in Shanghai in 2009,[16] in France from 2004 to 2007[6] and in Tunisia from 2005 to 2007,[13] to 20% in Iran between 2008 and 2009.[21] In Iran, it was reported to be 6.8% in the north from 2007 to 2010,[20] 20% in the south between 2008 and 2009[21] and 7.3% in the centre from 2005 to 2008.[15] Resistance to amoxicillin in our study was similar to other studies that were done in the north and the centre of Iran, but lower than the study done in the south of Iran.

In our study, no resistance was observed for tetracycline. In other studies, resistance to tetracycline ranged from no resistance in France between 2004 and 2007[6] to 38.1% in Iran from 2005 to 2008.[15] In Iran, it was reported to be 9% in the north from 2007 to 2010,[20] 3% in the south between 2008 and 2009[21] and 38.1% in the centre from 2005 to 2008.[15] Resistance to tetracycline in this study is lower than the studies performed in Iran. It is massively different from the study that was done in the centre of Iran. It is similar to the amount of resistance to tetracycline in France.

In this study, there was no relationship between age and resistances to clarithromycin, amoxicillin or tetracycline, but resistance to metronidazole was more common in the patients younger than 50 years age (p<0.05). An earlier

study reported no relationship between age and antibiotic resistance.[12]

In the present study, there was no significant difference between antibiotic resistance of H. pylori and gender. It is consistent with literature,[12] but there has been one study reporting resistance to metronidazole being higher in female patients.

Conclusion

Considering the high resistance to metronidazole, it is not recommended as a first-line drug. Using clarithromycin, amoxicillin and tetracycline in the H. pylori treatment regimen seems to be logical. Moreover, according to the sensitivity and specificity of RUT, it can be a suitable alternative method for histology.

Acknowledgments

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