Cohort Study

Evaluation of antibiotic resistance of Helicobacter pylori bacteria obtained from gastric biopsy samples: A cohort study

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ABSTRACT

Objective: Helicobacter pylori infection is associated with a wide range of gastrointestinal diseases and is very common in developing countries. Overuse and self-prescribed antibiotics have led to antibiotic resistance and failure of complete eradication of the bacterium. The aim of this study is to evaluate the antibiotic resistance of H. pylori from samples obtained from gastric biopsy.

Methods: In this descriptive-analytical study was performed on 205 patients' samples positive for H. pylori infection. Following H. pylori testing, the sample were cultured with different antibiotics to obtain data regarding the resistance. Demographic information of the patients such as age, sex, employment status, area of residence and patient-related factors such as reason for referral, and previous history of treatment were obtained and evaluated for the correlation with antibiotic resistance.

Results: In this study, the mean age of the subjects was 42.32 ± 16.65 years. The most common reason for referral of patients in the present study was epigastric pain in 49.3% (101 patients). Antibiotic resistance to amoxicillin was 46.8%, tetracycline was 41%, metronidazole was 33.2%, clarithromycin was 70.7%, levofloxacin was 36.1% and bismuth was 19.5%. Sex, age, type of living (rural or urban), employment, reason for referral and history of treatment was not associated with any antibiotic resistance, p > 0.05.

Conclusion: Our study showed that clarithromycin resistance is the most common in our population followed by amoxicillin and tetracycline. Excessive use of these antibiotics and self-prescription should be analyzed in future studies and public-awareness programs might be required.

1. Introduction

Helicobacter pylori (h. pylori) is a gram-negative bacterium with 4–6 polar flagella that grows under microaerophilic conditions. This bacterium is found in large numbers on the mucosal surface of the stomach and can continue to grow by penetrating the mucosal layer. It can interestingly infect gastric mucus for decades, despite acquired immune and inflammatory responses and gastric epithelial replacement [1].

The global prevalence of h. pylori infection is more than 50% and is common in young individuals in developing countries [2,3]. Poor health, poor water supply, and overcrowding are among the factors that increase prevalence. Humans are probably the only source of infection. Transmission occurs from person to person through oral and oral-fecal routes [2,4]. It can also be transmitted through the aerosol to the contents of the stomach in endoscopic wards [5]. This bacterium is known to cause chronic gastritis type B and most cases of gastritis and peptic ulcers are attributed to it. It is also closely related to adenocarcinoma and gastric lymphoma and is known as a risk factor for these neoplasms [6,7]. Gastric adenocarcinoma is the fourth leading cause of cancer in the world. H. pylori is more associated with gastritis in developed countries, which may lead to gastric ulcer and gastric carcinoma; while in developing countries it is more associated with chronic diarrhea, malnutrition, and underlying infections such as enteric infections (typhoid fever and cholera) [8–10].
Amoxicillin, tetracycline, metronidazole, and clarithromycin are commonly used in combination with proton pump inhibitors and bismuth salts to treat H. pylori infections. However, side effects and antibiotic resistance lead to treatment fail in number of patients [6,11]. Several culture methods are therefore used to detect the antibiotic susceptibility and resistance of this bacterium in order to treat infection effectively [12-14]. However, standardized methods of testing remain short in Iran [15-17].

The resistance of a bacterium to several antibacterial agents has become increasingly prevalent in Iran. The two main mechanisms of this phenomenon are the acquisition of several unrelated resistant genes and mutations in a single gene or gene set that cause resistance. The development of multidrug-resistant strains with multiple gene acquisition occurs during successive stages of gene transfer and environmental selection in areas with high antimicrobial use [18]. The pattern of antibiotic resistance varies geographically and is also determined by a number of other demographic factors [19].

The present study aimed to investigate the antibiotic resistance of Helicobacter pylori from samples obtained from gastric biopsy.

2 Methods

In this descriptive-analytical study, in 205 patients’ biopsy samples, we evaluated resistance of amoxicillin, tetracycline, bismuth, metronidazole, clarithromycin and levofloxacin against H. pylori with risk factors (independent variables), age, sex, employment status, treatment history and reason for referral. The sampling method was census and samples of all the patients who underwent biopsy in year 2019-2020 were examined.

The minimum sample size to evaluate antibiotic resistance with 95% confidence with significant difference of 0.07 between the resistance of different antibiotics and was estimated to be 205 patients using the following formula:

\[
\text{Sample Size} = \frac{z^2 \times p(1-p)}{d^2}
\]

where \(z\) is the standard normal deviate for a 95% confidence level, \(p\) is the estimated proportion and \(d\) is the margin of error.

Written consent was obtained from patients to participate in this research. Demographic information of patients including age, sex, reason for referral, etc. was collected through a checklist and used for statistical analysis. Patients with malignancy, those who had taken antibiotics and proton pump inhibitors in the last two weeks, and disagreed to participate in the study were excluded. The study population was divided into four age groups: under 30 years, 30-40 years, 41-50 years, and more than 50 years. Three biopsy samples were taken from each patient in the antrum. One sample was performed for rapid urease test (in one tube) and two samples (in one tube to increase the chance of positive culture). Sterile tubes containing 500 μl of physiological saline were used to transfer the samples. If the transfer time was estimated to be more than 3 h, the Stuart transfer site or the BHI (brain heart infusion) broth (Merck Millipore) was used [38].

In the laboratory, urease-positive samples were crushed in 500 μl of saline solution by hand homogenization device and 100 μl of Brucella agar medium containing 10% sheep blood and specific supplement with vancomycin, trimethoprim, and B amphoteric antibiotics (Millipore Sigma) was added. The pellets were placed at 37 °C for 5 days under microaerophilic conditions (in a jar containing Anaerocult® A or CO2 incubator). Identification and diagnosis of Helicobacter pylori was achieved using cellular characteristics in hot staining, colony morphology and bacteriological morphology, urease activity, catalase, and oxidase activity [39].

To describe the data, descriptive statistics such as graphs were used and to investigate the relationship between antibiotic resistance and independent variables logistic regression was used. SPSS and R software were used to describe and analyze the data. For comparison and obtaining relationship of the optimal variables, the obtained results were compared with a significance level of 0.05.

This study was approved by the Research Ethics Board of Lorestan University of (XXX).

Unique identifying number is: researchregistry7606.

The methods have been stated in accordance with STROCC 2021 guidelines.

3 Results

Of 608 samples evaluated for H. pylori infection, 205 samples were positive. The mean age of 205 patients included in the study was 42.52 ± 16.65 years (range: 12–90 years). As can be seen in Fig. 1, 29.8% (n = 61) of the patients were in the age range of 30 years and younger. In terms of gender distribution, 50.7% (n = 104) of the patients were male. In terms of residence, 99.5% (n = 204) were patients living in the city and in terms of employment status, 33.2% (n = 68) were self-employed patients. 25.4% (n = 52) were housewives, 20.5% (n = 42) were employed, 14.1% (n = 29) were students and 6.8% (n = 14) were retired.

Frequency distribution of clinical features of patients studied.

As can be seen in Fig. 2, the most common reason for referral of patients was epigastric pain in 49.3% (n = 101) patients. Also, the cause of referral was dysphagia in 12.2% (n = 25) of patients with, heartburn in 11.7% (n = 24), nausea and vomiting in 10.2% (n = 21) of patients, loss of weight and appetite in 6.8% (n = 14) of patients, gastroesophageal reflux disease in 2.5% (n = 5) and presence of blood in feces in 0.5% (n = 1) patients. In terms of treatment history, 9.8% (20 patients) had a history of treatment and 7.3% (15 patients) patients had a history of antibiotic use, for the treatment of Helicobacter pylori.

Evaluation of frequency distribution of antibiotic resistance and sensitivity.

As reported in Fig. 3, in terms of antibiotic resistance in the studied patients, 46.8% (n = 96) patients reported amoxicillin resistance and 53.2% (n = 109) patients reported amoxicillin-sensitive culture results. Also, in 41% (n = 84) of the culture results, tetracycline resistance was reported, and 59% (n = 121) cultures showed tetracycline sensitivity. 33.2% (n = 41) of patients had metronidazole resistance and 66.8% (n = 137) of patients had metronidazole-sensitive culture. In 29.3% (n = 60) of the patients, clarithromycin resistance was seen in the culture and 70.7% (n = 145) of the patients had clarithromycin-sensitive culture.

In terms of amoxicillin resistance and age, 36.8% (n = 35) patients had the greatest resistance and patients aged 41–50 years had the least resistance, 12.5% (n = 12). The results of chi-square test showed no statistically significant difference in different age groups in terms of amoxicillin resistance, p = 0.19. 52.1% (n = 50) females had amoxicillin resistance, which was also not significantly different from male gender, p = 0.49. 23.9% of the patients were employed and showed amoxicillin resistance whereas least was seen in retired patients, 6.2%. The difference was also not statistically significant, p = 0.22. Majority of patients with amoxicillin resistance were presented with epigastric pain, 44.8% whereas none presented with blood in feces had resistance to amoxicillin. Amoxicillin resistance was not significantly different among patients in terms of reason for referral, p = 0.18. Patients with (17.7%) and without history of treatment (82.3%) also did not differ in terms of amoxicillin resistance, p = 0.39.

In terms of age, tetracycline resistance was greatest in patients aged less than 30 years, 34.5% and was seen least in patients aged 41–50 years, 11.9%. The age groups of the patients were not significantly associated with tetracycline resistance, p = 0.4. Tetracycline resistance in male (54.7%) and female (45.3%) also did not differ significantly, p = 0.4. Among patients living in urban (98.8%) and rural (1.2%) setting, the difference was also not significant, p = 0.41. 31.1% patients with tetracycline resistance were self-employed whereas, least resistance was seen in retired patients, 8.3%. We did not find any significantly correlation between tetracycline resistance and employment status, p = 0.6. Majority of patients with tetracycline resistance were presented with epigastric pain, 50% whereas 11% patients presented with blood in feces had resistance to tetracycline. Tetracycline resistance was not significantly different among patients in terms of reason for referral, p = 0.93. Patients with (13.1%) and without history of treatment (86.9%)
also did not differ in terms of tetracycline resistance, *p* = 0.23.

Greatest resistance to metronidazole was seen in patients age between 31 and 40 years, 32.3% whereas least was seen in those aged 41–50 years, 11.7%. Metronidazole resistance was not significantly associated with age groups, *p* = 0.25. 44.2% females and 55.8% males had metronidazole resistance which was also not significantly different, *p* = 0.30. All the patients with metronidazole resistance were living in urban setting however, the difference was not significant compared to those living in rural setting, *p* > 0.99. Most of the patients were self-employed 32.3% and 8.8% patients were retired. Employment status of the patients was also not associated with metronidazole resistance, *p* = 0.81. 51.1% patients with epigastric patients and 1.4% with blood in feces showed resistance. The reason of referral was not correlated with metronidazole resistance, *p* = 0.53. 82.4% patients without and 17.6% patients with the history of treatment had metronidazole resistance which did not differ significantly, *p* = 0.11.

In terms of clarithromycin resistance, patients aged less than 30 years were the most, 35% and least were aged 41–50 years. The age groups of the patients did not differ significantly with the antibiotic resistance, *p* = 0.66. 63.4% females had clarithromycin resistance, which was also not significantly different from male gender, *p* = 0.14. 98.3% patients were living in urban areas and 0.8% were living in rural areas. The difference between the type of living was not significant among patients with clarithromycin resistance, *p* = 0.3. 38.3% of the patients were housewives and showed clarithromycin resistance whereas least was seen in retired patients, 5%. The difference was also
America, Turkey and Pakistan, the prevalence of Helicobacter pylori infection is over 80%, but in the Scandinavian countries and the United Kingdom, the prevalence is reported to be between 20 and 40% [21]. Examination of 608 samples obtained from gastric biopsy of patients, showed 205 positive cases indicating prevalence of 33.7%. The results of the present study were consistent with Yamazaki et al. [22]. The prevalence is also similar to that reported in Scandinavian countries and the United Kingdom.

In the present study, the mean age of the patients included was 42.32 years. The results of the present study were consistent with the results of the study of Haruma et al. [41].

The first antibiotic to be used in the first line of treatment for Helicobacter pylori was metronidazole. The widespread use of metronidazole in both Helicobacter pylori and other infections, such as parasitic infections, appears to cause the development of highly resistant isolates [23]. The drug as an external agent, and during the natural selection process causes the replacement of the sensitive majority population by the resistant minority population. In the present study, metronidazole resistance was reported to be 33.2%, whereas the average metronidazole resistance in Asia is 46.6%. Our results showed less than the average resistance to metronidazole in Africa (97.5%) and almost equal to the average resistance in Europe (34.2%). It was lower than different studies [24]. In the present study, no significant relationship was observed between any of the studied components such as age, sex, place of residence, reason for referral and history of treatment with metronidazole resistance. The results of the present study were consistent with the results of the cross-sectional cohort study by Savari et al., where among 191 patients metronidazole, clarithromycin, and tetracycline resistance was the highest [25].

Based on the prevalence of Helicobacter pylori resistance to amoxicillin in a cross-sectional study, including 185 patients, resistance to amoxicillin has been reported to be very low. The highest and lowest levels of resistance to amoxicillin in Iran are 28.6% in Tabriz and 1.6% in Tehran, respectively [26]. In the population of the present study, the resistance to Helicobacter pylori to amoxicillin was reported to be 46.8%, which indicates the high resistance of the bacteria to this compared to a randomized controlled trial by Caliskan et al., including 98 patients where amoxicillin and tetracycline resistance was the highest, respectively [27]. This can be due to the self-medication of various antibiotics, especially amoxicillin in the general population. In a cross-sectional study Shoita et al., reported that none of the h. pylori samples in their study were resistant to amoxicillin among 656 patients [28]. The resistance of h. pylori to amoxicillin in our study was lower than the than those reported in a prospective study by Rafeey et al., [29] which was 59%. The average resistance to tetracycline according to studies in Iran is 11.5% and like other antibiotics used in the treatment of Helicobacter pylori infections, tetracycline resistance is increasing. According to the studies, the highest value of resistance is reported in Tehran, 38.1% and the lowest level of resistance is reported to be zero in another study conducted in Tehran, which necessitates further investigation in this area [30]. In the present study, the resistance of h. pylori to tetracycline was 41%, which is higher than a single center prospective study conducted by Almeida et al., on 180 patients [31]. This rate is 0.5% in Europe, 65.3% in Africa and 13.9% in Asia. The use of clarithromycin in the treatment of diseases is small, however, in studies, high amounts of resistance to clarithromycin are reported [32]. One of the causes of clarithromycin resistance is the use of macrolides. Resistance is higher in children due to the administration of macrolides for the treatment of respiratory diseases [33]. According to studies, the average resistance to clarithromycin is 17%, but in the present study, the results show that h. pylori resistance to clarithromycin in the population 70.7%, which is much higher than the results of similar studies. Clarithromycin resistance rates are 25.1% in Europe, 24% in Asia, 7.2% in the United States, and 50.8% in Africa [34]. In the present study, no significant relationship was observed between clarithromycin resistance and the studied variables. Clarithromycin is one of the macrolide antibiotics that has not been used much in Iran in past years due to its high cost, but today it is available due to the production of this drug in the country and

4. Discussion

Helicobacter pylori infection is one of the most important causes of gastrointestinal diseases. In-vitro studies have shown that some probiotics have shown good inhibitory effects on h. pylori and have even been able to alleviate the effects of infection in patients [20].

In terms of geographical prevalence of h. pylori in Japan and South America, Turkey and Pakistan, the prevalence of Helicobacter pylori infection is over 80%, but in the Scandinavian countries and the United

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Fig. 3. Frequency of antibiotic resistance and sensitivity in the studied patients.
is widely used, especially in the treatment of H. pylori infections. The subject may be the reason for the high resistance to this antibiotic in the present study.

Fluoroquinolones (such as ciprofloxacin, moxifloxacin, trovafloxacin, and levofloxacin) are antibiotics that exert their antibacterial activity through the DNA enzyme esterase [35]. Most Helicobacter pylori isolates are sensitive to fluoroquinolones, but resistance to fluoroquinolones among these strains is increasing. Initial resistance to fluoroquinolones has been reported ranging from 2 to 22% in different countries [36]. Resistance in countries with high consumption of levofloxacin is relatively high (>19%) [37]. It was also found that the resistance of H. pylori against this antibiotic has increased over the past 21 years (1997–2017). The prevalence of levofloxacin resistance is higher in Asia (25.8%), South America (21.33%) compared to Europe and Africa (less than 15%) [38,39].

Different results in determining the antibiotic susceptibility of Helicobacter pylori in the present study and in other studies can be related to various factors such as improper choice of antibiotic, overse of antibiotics, inaccuracy in the time required for treatment, inability of each laboratory to cultivate this bacteria followed by antibiogram testing for it and most importantly non-compliance with standard conditions, especially in the various stages of antibiogram testing. Our study does not report treatment outcomes or association of antibiotic resistance with previous use of antibiotics, which of great importance in these patients.

5Conclusion

The results of this study show that resistance to common antibiotics in drug regimens against Helicobacter pylori is increasing. The resistance of H. pylori to clarithromycin, amoxicillin and tetracycline is higher than other antibiotics and is not correlated with demographic and patient-related variables. Culture and susceptibility testing are necessary to determine the patterns of drug resistance of this bacterium in different geographical areas before the treatment.

Provenance and peer review

Not commissioned, externally peer-reviewed.

Conflicts of interest

The authors deny any conflict of interest in any terms or by any means during the study.

Sources of funding

No funding was secured for this study.

Ethical approval

All procedures performed in this study involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Consent

Not applicable.

Author contribution

Dr. Salehe Azadbakht and Dr. Siamak Alihoseini Fard: conceptualized and designed the study, drafted the initial manuscript, and reviewed and revised the manuscript.

Dr. AliReza Moayyedkazemi: Designed the data collection instruments, collected data, carried out the initial analyses, and reviewed and revised the manuscript.

Dr. Salehe Azadbakht and Dr. Setareh Soroush: Coordinated and supervised data collection, and critically reviewed the manuscript for important intellectual content.

Registration of research studies

Name of the registry: Tehran Islamic Azad University of Medical Sciences.

Unique Identifying number or registration ID: IR.LUMS.REC.1400.088.

Hyperlink to the registration (must be publicly accessible): https://ethics.research.ac.ir/ProposalCertificateEn.php?id=203059&print=true

Guarantor

Siamak Alihoseini Fard.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jamsu.2022.103824.

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