

Prevalence of metronidazole resistant *Helicobacter pylori* strains among Chinese peptic ulcer disease patients and normal controls in Hong Kong

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Abstract

Background—A study was conducted to evaluate the prevalence of metronidazole resistant *Helicobacter pylori* strains among the Chinese in Hong Kong. The efficacy of the triple therapy that contains metronidazole as one of the anti-microbial agents in eradication of the metronidazole susceptible and the metronidazole resistant strains was also assessed.

Methods—Culture for *H pylori* was attempted from antral biopsy specimens of 70 peptic ulcer and 51 control subjects. Successfully cultured *H pylori* strains were tested for metronidazole susceptibility. Twenty six peptic ulcer disease subjects who had received a course of triple therapy were also reassessed four to six weeks later for successful eradication of *H pylori* infection.

Results and conclusions—*H pylori* was successfully cultured from antral biopsy specimens in 69 of 80 (86%) of the infected subjects. The overall metronidazole resistance rate was 53.5% (37 of 69). There was a significantly higher metronidazole resistance rate among *H pylori* isolates from the asymptomatic controls (20 of 25) than the peptic ulcer disease subjects (17 of 44) ($p=0.0007$). Twenty three of 32 (73%) women and 14 of 37 (38%) men harboured the metronidazole resistant strains. There was no sex or age difference as far as the prevalence of metronidazole resistant strains were concerned within each study group. Pre-treatment metronidazole susceptible *H pylori* were significantly more likely to respond to the triple therapy used than those with the metronidazole resistant ones (14 of 15 *v* five of 10) ($p=0.021$).

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Keywords: *Helicobacter pylori*, metronidazole susceptibility, Chinese.

Treatment of peptic ulcer disease has been revolutionised ever since the demonstration of significantly lower ulcer, both duodenal and gastric ulcer, relapse rates after successful *Helicobacter pylori* eradication.¹⁻⁶ This led to the recent recommendation by the National Institute of Health Panel that anti-*H pylori* therapy should be considered for every patient with peptic ulcer disease when it is associated with *H pylori* infection.⁷ Such a policy is more

or less adopted by most gastroenterologists worldwide. The most widely used combination therapies currently consist of either a proton pump inhibitor or bismuth compound together with two antibiotics. One of the antibiotics used is a nitroimidazole, for example, metronidazole. Previous reports showed that the overall *H pylori* eradication rate depended significantly on the organism's susceptibility towards metronidazole,⁸⁻¹² with a significantly lower eradication rate in those with the metronidazole resistant strains than those with the metronidazole susceptible ones. It has been demonstrated in a recent survey by Glupczynski *et al*¹³ that natural resistance of *H pylori* against nitroimidazoles occurs between 7% (Madrid) to 49% (Athens) in Europe. In contrast, it is approximately 80% in the developing countries for example in Africa.^{14 15} Little, however, is known about the prevalence of metronidazole resistant *H pylori* strains among the Chinese in Hong Kong. We have therefore conducted a study among peptic ulcer disease patients and a group of healthy volunteers to report in the prevalence of metronidazole resistant *H pylori* strains and to assess the influence of pre-treatment metronidazole resistance on the final eradication rate by combination therapy that includes metronidazole as one of the antimicrobial agents.

Methods

Subjects

Seventy patients with peptic ulcer disease were entered into this study. Twenty six (25 duodenal ulcer and one gastric ulcer) cases were given anti-*H pylori* therapy (16 males, mean age 47.8 years; 10 females, mean age 52.7 years), and the other 44 (38 duodenal ulcer and six gastric ulcer) cases (28 males, mean age 44.7 years; 16 females, mean age 56.4 years) received either omeprazole or ranitidine as anti-ulcer therapy. Fifty one healthy volunteers (23 males, mean age 48.1 years; 28 females, mean age 60.6 years) recruited from medical exhibitions were included as controls in this study. The 26 peptic ulcer disease patients received omeprazole 20 mg twice daily (for six weeks) and metronidazole 300 mg four times daily (for 14 days) in combination with either clarithromycin 250 mg four times daily (13 cases) or amoxicillin 500 mg four times daily (13 cases) (for the first 14 days). Repeat endoscopy and gastric biopsies was performed four to six weeks on 25 of these

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26 patients (one defaulted from follow up) after completion of anti-*H pylori* treatment to reassess the eradication rate by urea broth test and histological examination.

Endoscopy and biopsy specimens

All the above patients underwent upper gastrointestinal endoscopy after local lignocaine throat spray. Six biopsy specimens were taken from the antrum, two for culture and sensitivity testing, two for urea broth test,¹⁶ and two for histological examination¹⁷ as described before. Only two specimens were taken from the corpus of the stomach for histological examination.

Diagnosis of *H pylori* infection

A positive diagnosis of *H pylori* was made when at least two of the three tests, the urea broth test, histology, or microbiology were positive. Eradication of *H pylori* after triple therapy was confirmed by a negative urea broth test and histology showing absence of *H pylori* both in the antrum and in the corpus.

H pylori culture and sensitivity

Antral biopsy specimens obtained from these patients were totally embedded into Stuart transport medium (Oxoid, UK), stored at 4°C, and sent to the microbiology laboratory within two hours. Samples were ground by tissue grinder in 0.5 ml sterile saline and the homogenate was inoculated onto a Dent's selective medium (Oxoid, UK), which consisted of columbia blood agar base, 7% laked horse blood, and mixtures of antimicrobial and antifungal agents (vancomycin 10 mg/l, trimethoprim 5 mg/l, cefsulodin 5 mg/l, and amphotericin B 5 mg/l). They were incubated for up to seven days at 37°C under microaerophilic conditions (80% O₂, 15% N₂, and 5% CO₂) as described before.¹⁸ *H pylori* colonies were confirmed by a positive urease, oxidase, and catalase test together with the identification of a Gram negative spiral looking organism on microscopy. Metronidazole susceptibility was determined by using the modified Kirby-Bauer disc diffusion procedure as described by DeCross *et al.*¹⁸ Briefly, *H pylori* colonies were harvested into brain heart infusion broth (Oxoid, UK) plus 10% horse serum to produce a cloudy suspension of McFarland no 3 to 4. A sterile swab was dipped into the *H pylori* suspension, squeezed out, and streaked evenly across a 5% horse blood agar plate. Duplicate plates were made on each isolate. They were briefly dried, and then a 6 mm disc containing 5 µg/ml metronidazole was added to each plate before they were subjected to microaerophilic incubation as described above for seven days. The inhibition zone diameters were recorded then. An inhibition zone diameter of >15 mm was regarded as metronidazole susceptible,¹⁸ and resistant to metronidazole if it was less than 15 mm.

Statistical analysis

The χ^2 and Fisher exact tests were used to compare the difference in percentage of metronidazole resistant *H pylori* strains between the peptic ulcer disease group and the healthy volunteers. Furthermore, they were also used to test the difference in the eradication rates between the metronidazole susceptible and the metronidazole resistant *H pylori* strains. The Mann-Whitney U test was used to show if there was any age difference between men and women within the peptic ulcer and the control groups. A p value of <0.05 was considered significant.

Results

H pylori culture rate and the prevalence of metronidazole resistance (Table)

A total of 80 (44 peptic ulcer and 25 healthy) subjects were found to be *H pylori* positive by a combination of urea broth test and histology. Sixty nine (86%) were successfully cultured by the techniques described above. Metronidazole resistance was found in 37 of 69 (53.5%) and metronidazole susceptible in 32 of 69 (46.5%) of the primary isolates (Table). Among the peptic ulcer disease group, 17 of 44 (38.6%) were found to be metronidazole resistant. However, in the healthy subjects, 20 of 25 (80%) were metronidazole resistant. There was significantly higher metronidazole resistant *H pylori* strains present in the healthy than in the peptic ulcer disease group (p=0.0007). However, no significant difference in the metronidazole resistant *H pylori* strains was seen between the men and the women in either the peptic ulcer disease group (p=0.052) or the healthy subjects (p=0.19). But the overall prevalence of metronidazole resistant *H pylori* strains was significantly higher in women (23 of 32, 73%) than in men (14 of 37, 38%) (p=0.005). There was no significant age difference between those carrying the metronidazole resistant strains and those carrying the metronidazole susceptible ones (p=0.093). However, women (mean age 58.1 years) were significantly older than men (mean age 45.8 years) in those with a positive *H pylori* culture (p=0.008). In the culture negative group, there was no age difference between the men (mean age 48.3 years) and the women (mean age 57 years) (p=0.082). Similarly, there was no age difference between the men and the women both among the peptic ulcer disease and the

Metronidazole susceptibility of the 69 *Helicobacter pylori* isolates

	Peptic ulcer disease (n=44)	Controls (n=25)
Metronidazole resistant		
Males	8	6
Females	9	14
Total	17	20
Metronidazole sensitive		
Males	20	3
Females	7	2
Total	27	5

healthy volunteer groups in the *H pylori* culture positive subjects.

Differential H pylori eradication rates between metronidazole susceptible and the metronidazole resistant groups

Nineteen of 25 duodenal ulcer patients who received triple therapy were found to be negative for *H pylori* infection. There was a significantly higher *H pylori* eradication rate in those with metronidazole susceptible strains (14 of 15; 93%) than the metronidazole resistant strains (five of 10; 50%) ($p=0.021$). For metronidazole resistant strains, there was no difference in the eradication rate between the amoxicillin (two of five) and the clarithromycin (three of five) containing anti-*H pylori* regimens.

Discussion

Metronidazole resistant *H pylori* strains occurred in about half of the culture positive subjects in this study. However, the prevalence of metronidazole resistant *H pylori* was disproportionately higher in the healthy subjects than in the peptic ulcer disease patients, being roughly twice as frequent in the former. The prevalence of metronidazole resistant *H pylori* strains in Hong Kong is somewhere between that of the developed and the developing countries.¹³⁻¹⁵ Interestingly, those carrying the metronidazole resistant strain were not significantly older than those carrying the metronidazole susceptible ones. Although it has been noticed in previous studies that there is increasing *H pylori* infection carrier rates with age, age however in itself may not be a determining factor in metronidazole resistance property as borne out by this study. On the other hand, a significantly higher rate of metronidazole resistant *H pylori* among the women, whether in this study or the others,⁸⁻¹³ could be perhaps accounted for by the previous history of nitroimidazole ingestion for pelvic inflammatory diseases. Unlike the developing countries such as Africa, metronidazole is not so widely used in Hong Kong because there is a much lower rate of giardiasis or amoebiasis. As a result, there is a much lower prevalence of metronidazole resistant *H pylori* strains in Hong Kong. The fact that there is a significantly lower metronidazole resistant *H pylori* rate among the peptic ulcer disease patients compared with the healthy subjects is encouraging for clinicians. This is supported by the high *H pylori* eradication rates (about 80%) achieved by using combination therapies that consist of metronidazole as one of the antimicrobial agents^{2 6 19} among the Chinese in Hong Kong. It has been reported in a number of previous studies that the metronidazole resistant *H pylori* strain is much more difficult to eradicate.⁸⁻¹² This is further supported by our study. The choice of combination therapy may have to be tailored according to the susceptibility of the organism towards metronidazole.²⁰⁻²³

It has been suggested by some that routine culture and sensitivity against metronidazole

should be performed in cases where anti-*H pylori* therapy is contemplated so that a much higher eradication rate could be achieved by using the appropriate combination therapy.²⁰ This approach may not be as practical as it seems because it is quite a tedious and time consuming process. On average, it takes about 10 working days to confirm the susceptibility of the organism against metronidazole. This approach will undoubtedly confer delay in initiating therapy for those requiring treatment. The added problem to this is the false negative culture result, which occurs between 10-20%. We would suggest that triple therapy consisting of either a bismuth compound or omeprazole in combination with amoxicillin and metronidazole be given to ulcer patients while waiting for metronidazole resistance results. This combination therapy will (a) heal the ulcer effectively, and (b) satisfy the patients who require active therapy without any delay. This combination, albeit with a lower eradication rate in those with metronidazole resistant strains, would not induce further antibiotic resistance in the organism because no amoxicillin resistant *H pylori* strains have been reported yet. If subsequent results show that the organism is indeed metronidazole resistant and there is a failure of eradication by confirmatory testing at least four weeks after completion of the antibiotics, alternative combination therapy such as reported by Logan *et al*²⁰ could be tried on another occasion. This approach may be the best compromise until an ideal combination therapy has been found that can eradicate *H pylori* effectively irrespective of metronidazole susceptibility.

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