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Refractory *Helicobacter pylori* gastritis: The hidden predictors of resistance

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ABSTRACT

Objectives: Failure of *Helicobacter pylori* eradication is documented in 20% of patients. Some patients show a negative faecal antigen test (FAT) with persistent symptoms after therapy. The aim of this study was to detect occult *H. pylori* infection in patients with persistent symptoms despite FAT negativity following therapy.

Methods: A total of 200 symptomatic patients presenting with dyspepsia and positive FAT were treated with *H. pylori* triple therapy for 2 weeks. Refractory patients received levofloxacin-based salvage therapy. Upper gastrointestinal endoscopy was performed for patients with persistent symptoms despite negative FAT after salvage therapy. Gastric biopsies were exposed to rapid urease test and RFLP-PCR for clarithromycin resistance in domain V of 23S rRNA (2142/2143 point mutations) as well as culture and antimicrobial susceptibility testing (AST).

Results: A total of 136 patients responded to classic triple therapy with negative FAT, and 15 patients showed persistent symptoms with positive FAT and received salvage therapy. The remaining 49 patients showed persistent symptoms despite negative FAT, therefore gastric biopsies with rapid urease test were performed. Clarithromycin resistance was confirmed in 12/49 patients (24.5%). Cultures were most commonly susceptible to norfloxacin ($n = 18$), moxifloxacin ($n = 13$), doxycycline ($n = 11$) and amikacin ($n = 8$). Non-responders with negative FAT had moderate or severe fatty liver disease (26.5% and 32.7%, respectively), 40.9% had hepatitis C virus (HCV) infection, and they had significantly higher HOMA-IR and HbA1c.

Conclusion: Diabetes mellitus, HCV and non-alcoholic fatty liver disease predispose to refractory *H. pylori* requiring culture and AST.

1. Introduction

Helicobacter pylori is considered a public-health problem as it is involved in the development of duodenal and gastric ulcers, gastric cancer, gastric mucosa-associated lymphoid tissue (MALT) lymphoma and gastric adenocarcinoma [1].

H. pylori is a flagellate, Gram-negative, spiral-shaped bacterium with microaerophilic behaviour [2,3]. Infection with *H. pylori* is commonly acquired during childhood and the methods of transmission are mainly person-to-person and interfamilial [4]. Acid secretion is increased in antrum-predominant gastritis, whereas corpus-predominant gastritis is characterised by reduced acid secretion and the development of intestinal metaplasia, glandular atrophy and hypochlorhydria [5].

Pathogenic factors of *H. pylori* are the presence of flagella, urease enzyme production, mucosal adherence by bacterial adhesin (BabA), mediation of tissue injury by lipopolysaccharide that interacts trefoil factor 1 to promote colonisation, effector protein cytotoxin-associated gene A (CagA), vacuolating cytotoxin (VacA), outer inflammatory protein (OipA) and duodenal ulcer-promoting protein A (DupA).

In addition, *H. pylori* reduces the production of heat shock proteins leading to immune evasion with long-term colonisation [6]. The predominant immunological mechanisms in *H. pylori* infection are the Th1 immune response triggered by interleukin 12 (IL-12) and IL-18 following *H. pylori* antigen presentation and recognition by gastric epithelial cells, dendritic cells and macrophages with enhanced production of Th1-dominated cytokines such as tumour necrosis factor-alpha (TNFα), IL-1β and interferon-gamma (IFNγ) [7].

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Mechanisms of refractory *H. pylori* are the presence of clarithromycin- and metronidazole-resistant strains (30% and 30–60% of cases, respectively) [8], previous macrolide experience, patient non-compliance, and failure of adequate acid suppression that promotes *H. pylori* to become more susceptible to antimicrobial therapy [9].

Although classic triple therapy is effective, it is associated with treatment failure in 20–30% of patients [10] or even higher in real-life clinical practice [11,12].

H. pylori shows oncogenic behaviour. Eradication reduces the gastric cancer incidence by 39% and reverses atrophic gastritis in 50%, therefore confirmed *H. pylori* infection should be treated even in asymptomatic patients [13,14].

Poor glycaemic control had been found to increase the risk of persistence and the carcinogenic effect of *H. pylori*. Ikeda et al. reported a greater risk of gastric cancer in those with high haemoglobin A1c (HbA1c) ≥6% and co-existent *H. pylori* infection [15].

Chronic hepatitis C virus (HCV) infection is associated with *H. pylori* in nearly 40% of patients. Its possible localisation in the gastric microenvironment might induce the persistence of *H. pylori* and may trigger the occurrence of gastric non-Hodgkin's lymphoma [16].

The aim of the current study was to determine the incidence of occult *H. pylori* infection in gastric biopsies obtained by upper gastrointestinal endoscopy in patients with persistence of symptoms following classic triple therapy despite a negative faecal antigen test (FAT).

2. Materials and methods

2.1. Patient selection

This was an observational study conducted from May 2016 to June 2018 in the Gastroenterology Outpatient Clinic and Endoscopy Unit of the Internal Medicine Department of Zagazig University Hospital, which is a tertiary referral centre in Zagazig, Egypt. A total of 200 patients with epigastric pain and dyspepsia with positive FAT for *H. pylori* were treated with tripe therapy of esomeprazole 40 mg once daily, amoxicillin 1 g twice daily and clarithromycin 500 mg twice daily for 2 weeks.

Patients were evaluated by taking a complete history, physical examination and symptom assessment by the Quality of Life in Reflux and Dyspepsia (QOLRAD) questionnaire, which is composed of five domains: emotional distress (score, 6–41); sleep disturbance (score, 5–35); vitality (score, 3–21); food/drink problems (score, 6–40); and social/physical functioning (score, 5–35), giving a total possible score of 25–172, with a lower value indicating a more severe impact on daily activities [17].

Patients with persistent symptoms as assessed by QOLRAD and persistently positive FAT performed 6 weeks after finishing first-line therapy underwent abdominal ultrasound to rule out more serious symptomatic disease and received salvage therapy that included a 7-day course of 500 mg levofloxacin once daily, 1000 mg amoxicillin twice daily and 40 mg esomeprazole once daily (Fig. 1).

Inclusion criteria were patients diagnosed with *Helicobacter*-induced gastritis with persistent symptoms despite negative FAT after first-line therapy. Exclusion criteria included gastritis other than due to *Helicobacter*, gastric malignancy, recent use of antibiotics (within 6 weeks) or proton pump inhibitors (PPIs) (within 2 weeks), and active gastrointestinal bleeding.

The study was approved by the Ethical Review Board of the Faculty of Medicine of Zagazig University. Written informed consent was obtained from each patient included in the study. The study protocol conformed to ethical guidelines of the 1975 Declaration of Helsinki as reflected in a priori approval by the institution's Human Research Committee.

2.2. Biochemical investigations

2.2.1. Laboratory investigations

The following laboratory investigations were performed: liver and kidney function tests; complete blood count; fasting and postprandial blood glucose; HbA1c level; and fasting insulin, which was measured quantitatively by electrochemiluminescence immunoassay with a cut-off value 8.64 μIU/mL.

Insulin resistance was calculated by Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) using the following formula: fasting glucose (mg/dL) × fasting insulin (μIU/mL)/405; a value >2 indicates insulin resistance [18,19].

2.2.2. Association with other infections

The possible association of other infections in resistant cases of *H. pylori* was determined by detection of HCV antibody for HCV infection and hepatitis B surface antigen (HBsAg) for hepatitis B virus (HBV) infection. Cases with positive serology underwent quantitative PCR.

2.2.3. *Monoclonal* H. pylori *faecal antigen test (FAT)*

Stool samples (100 μL) were tested using enzyme immunoassay kits (ACON Laboratories Inc., San Diego, CA, USA) according to the manufacturer's instructions. Positive *H. pylori* infection status was determined as an *H. pylori* stool antigen ≥55 ng/mL. The inter- and intra-assay coefficients of variation of the assay were both <5%. The test was performed 6 weeks after first-line or salvage therapy.

2.3. Abdominal ultrasound

Patients with refractory *H. pylori* were examined for other causes of dyspepsia and abdominal pain such as fatty liver disease, liver cirrhosis, gallstones and pancreatic disorders.

2.4. Upper gastrointestinal endoscopy

A minimum of four biopsy specimens were obtained from the proximal and distal stomach to establish histological *H. pylori* eradication, as infection mostly had a patchy localisation.

2.5. Rapid urease test

The rapid urease test, also know as the CLO (*Campylobacter*-like organism) test, detects the presence of urease in the gastric mucosa. The rapid urease test was performed during endoscopy and the tissue was placed into a medium containing urea and a colour indicator; yellow (negative) and red (positive) discolorations were assigned. The rapid urease test was performed for diagnosis and after 4 weeks of termination of culture and antimicrobial susceptibility testing (AST)-based therapy.

2.6. Histopathology

Histopathology was performed to provide information about the degree of inflammation (mononuclear inflammatory cell and neutrophil infiltrates) and associated atrophic gastritis, intestinal metaplasia, dysplasia and gastric cancer. Biopsies were fixed in 10% formalin, followed by haematoxylin and eosin (H&E) and Giemsa staining. Grading was done according to the updated Sydney System, which depends on the intensity of mononuclear inflammatory infiltrates within the lamina propria: absent inflammation

(Grade 0); mild inflammation (Grade 1); moderate inflammation (Grade 2); and severe inflammation (Grade 3) [20].

2.7. DNA extraction

DNA was extracted from biopsies using a Genomic DNA Purification System (QIAamp® DNA Mini Kit; QIAGEN, Hilden, Germany). The following primers were used for amplification: forward primer, 5′-AAGCTTTTAGGGGTGT T AGGGGTTT-3′); and reverse primer, 5′-AA GCTTA CTTTCTAA CA CTAACGC-3′) [21].

Amplification and restriction fragment length polymorphism (RFLP) PCR were performed for clarithromycin resistance in domain V of 23S rRNA (2142/2143 point mutations). A 1400-bp fragment from an internal region of the 23S rRNA gene was amplified and was exposed to digestion with the restriction enzymes *Bsa*I and *Mbo*II. For the *Bsa*I enzyme, if the gene is wild-type, digestion will result in two fragments of 1000 bp and 400 bp. If the A2143G point mutation exists, the enzyme will produce three fragments of 700, 400 and 300 bp. For the *Mbo*II enzyme, if the gene is wild-type, the 1400-bp fragment remains without digestion; however, in case of A2142G point mutation, the fragment will be digested into two fragments overlapping each other on gel electrophoresis.

2.8. Culture and antimicrobial susceptibility testing (AST)

Biopsy specimens were incubated for 3 h at 37 °C in a sterile Eppendorf tube containing 1 mL of 2% trypsin solution. At the end of the first hour of incubation, the trypsin-digested biopsy sample was separated from the trypsin solution by washing in a centrifuge for 3 min, the supernatant was discarded and the sample was subsequently washed with 1 mL of 0.9% sodium chloride. The processed tissue was then seeded on Mueller–Hinton agar containing sheep blood and was incubated at 35 °C in a microaerophilic (5% O_2, 10% CO_2, 85% N_2) environment and observed for growth after 2 days and then daily for 10 days before confirming negativity.

Helicobacter pylori was identified by Gram stain as well as by oxidase, catalase and urease tests.

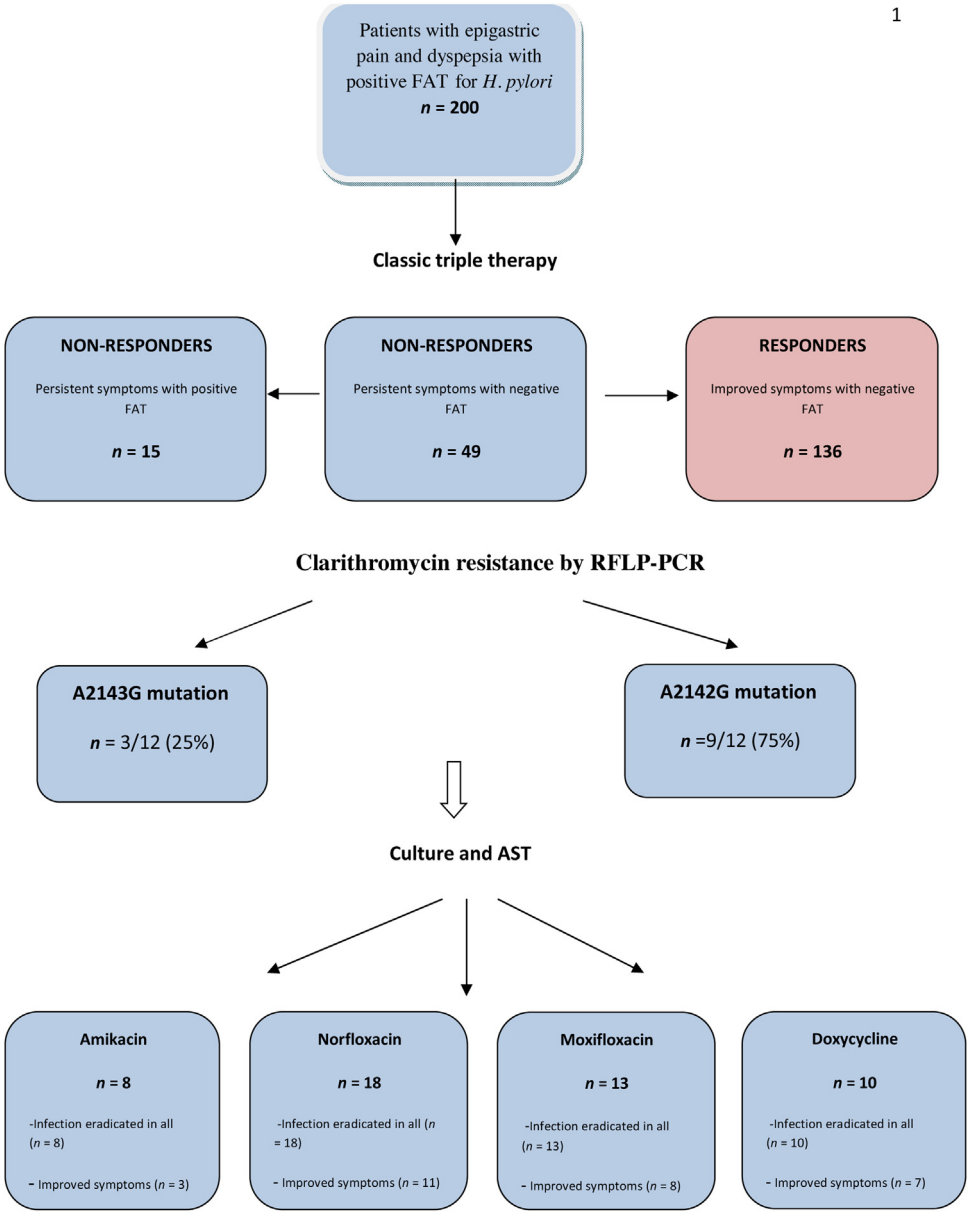

Fig. 1. Flow chart of the study population. FAT, faecal antigen test; RFLP, restriction fragment length polymorphism; AST, antimicrobial susceptibility testing.

Various antibiotics, including clarithromycin, amoxicillin, metronidazole, doxycycline, rifamycin, levofloxacin, moxifloxacin, norfloxacin and amikacin, were tested for susceptibility. Gradient strips were used to determine minimum inhibitory concentrations (MICs) of amoxicillin, metronidazole and clarithromycin. The MIC cut-off values for clarithromycin (susceptible, ≤0.25 μg/mL; resistant, ≥1 μg/mL) were taken from the Clinical and Laboratory Standards Institute (CLSI). The other agents do not have established CLSI interpretive criteria and the following cut-offs were used: amoxicillin, susceptible, ≤0.12 μg/mL, resistant, >0.5 μg/mL; and metronidazole, susceptible <8 μg/mL, resistant, >8 μg/mL.

The disk diffusion method was used for doxycycline, rifamycin, levofloxacin, moxifloxacin, norfloxacin and amikacin to easily detect the lowest MIC after incubation for 48 h at 37 °C in a microaerobic atmosphere. The following cut-off diameters (in mm) were used: doxycycline, resistant <17, susceptible >19; rifamycin, resistant <14, susceptible >19; levofloxacin, resistant <17, susceptible >20; amikacin, resistant <15, susceptible >19; moxifloxacin, resistant <16, susceptible >20; and norfloxacin, resistant <18, susceptible >26.

2.9. Statistical analysis

Data were analysed using IBM SPSS Statistics for Windows v.20 (IBM Corp., Armonk, NY, USA). The sample size was determined according to an equation that utilised population size, confidence level (%) and margin of error (%). Continuous variables were expressed as the mean ± standard deviation. Categorical variables were analysed using the χ^2 test, and continuous variables were analysed using the appropriate *t*-test. Correlation analysis and logistic regression were performed to detect variables independently associated with persistence of *H. pylori* infection. A *P*-value of <0.05 was considered statistically significant.

3. Results

3.1. Patient characteristics

A total of 200 patients were evaluated after reporting symptoms of dyspepsia (*n* = 200; 100%), abdominal pain (*n* = 56; 28.0%), gastroesophageal reflux (*n* = 130; 65.0%) and early satiety after feeding (*n* = 87; 43.5%) and were enrolled following confirmation of *H. pylori* gastritis by FAT. The patient baseline characteristics and demographics are shown in Table 1, and their baseline QOLRAD scores are shown in Table 2.

Of the 200 enrolled patients, 136 responsive patients (68.0%) showed improved QOLRAD score from baseline (Table 2) with documented FAT negativity, indicating bacterial eradication. Fifteen patients (7.5%) showed persistent symptoms with a non-significant change of QOLRAD from baseline (Table 2) and a positive FAT at 6 weeks after therapy. These patients were treated with 7 days of salvage therapy and 6 weeks after treatment all of the patients showed symptomatic improvement in QOLRAD from baseline (Table 2) with documented FAT negativity.

The remaining 49 patients (24.5%) showed persistent symptoms with non-significant change of QOLRAD from baseline (Table 2) despite FAT negativity. Gastric biopsies were taken from the suspicious areas (at least four biopsies from the antrum, corpus and incisura along the greater curvature) by upper gastrointestinal endoscopy and were immediately exposed to rapid urease test, which was positive in all 49 patients. Histopathology revealed chronic gastritis, mainly antral (*n* = 23) or combined antral and corporeal (*n* = 26), with metaplasia in 6 patients. Sydney grading revealed G2 in 25 patients and G3 in 24 patients.

Clarithromycin resistance detection by RFLP-PCR in domain V of 23S rRNA (2142/2143 point mutations) was positive in 12/49 patients (24.5%); 9/12 patients had the A2142 G mutation and 3/12 patients had the A2143G mutation.

Differences in characteristics between the three subgroups (responders, non-responders with positive FAT and non-responders with negative FAT) are shown in Table 3. Females were more predominant among responders, and age was significantly higher in non-responders. Smoking, diabetes mellitus and previous macrolide exposure were significantly more frequent in non-responders. Moreover, 40.9% and 10.2% of non-responders with negative FAT had co-infection with HCV and HBV, respectively (Figs. 2 and 3).

Fatty liver disease was diagnosed by ultrasound in 85 patients (42.5%). Of the 49 non-responders with negative FAT, 13 (26.5%) and 16 (32.7%) had moderate and severe fatty liver disease, respectively (Fig. 3). Non-responders had higher uric acid, HOMA-IR and HbA1c values compared with responders.

Culture and AST were applied to gastric biopsies from non-responders with negative FAT and showed that susceptibility was most common to norfloxacin (*n* = 18), moxifloxacin (*n* = 13), doxycycline (*n* = 11) and amikacin (*n* = 8). Salvage therapy was given as the effective antibiotic revealed by culture and AST, combined with nitazoxanide owing to its activity against aerobic and anaerobic bacteria as well as anaerobic protozoa and its potent effect on metronidazole-resistant strains, plus high-dose PPI. Thus, therapy was initiated as follows: norfloxacin 400 mg twice daily, nitazoxanide 500 mg twice daily and 40 mg esomeprazole once daily for 10 days (*n* = 18); moxifloxacin 400 mg once daily, nitazoxanide 500 mg twice daily and 40 mg esomeprazole once daily for 10 days (*n* = 13); doxycycline 100 mg twice daily, nitazoxanide 500 mg twice daily and 40 mg esomeprazole once daily for 10 days (*n* = 10); and amikacin according to age (<60 years) and creatinine clearance (>90 mL/min), 250 mg intravenous every 12 h for 5 days, nitazoxanide 500 mg twice daily and 40 mg esomeprazole once daily for 2 weeks (*n* = 8).

No side effects were reported and all patients underwent upper gastrointestinal endoscopy 6 weeks after treatment termination to take gastric biopsies for rapid urease test, which was negative in all patients. Symptomatic improvement with significant improvement of QOLRAD score (Table 2) was recorded in 29/49 patients (59.2%) after therapy, mainly in the norfloxacin-based therapy group (*P* = 0.01).

Variables correlated with persistence of *H. pylori* were body mass index (BMI) (*r* = 0.570, *P* = 0.001), age (*r* = 0.640, *P* = 0.000), HOMA-IR (*r* = 0.802, *P* = 0.000), previous macrolide exposure (*r* = 0.531, *P* = 0.001), severity of fatty liver disease (*r* = 0.515, *P* = 0.001) and HCV positivity (*r* = 0.350, *P* = 0.003).

Table 1
Patient baseline characteristics and demographics as well as laboratory and ultrasound characteristics of the study population.

Characteristic	*n* (%)[a]
Male/female (*n*)	134/66
Age (years) (mean ± S.D.)	39.1 ± 8.5
BMI (kg/m^2) (mean ± S.D.)	26.6 ± 2.7
Smoking	62 (31.0)
Diabetes mellitus	44 (22.0)
Previous macrolide exposure	48 (24.0)
HCV	38 (19.0)
HBV	13 (6.5)
Fatty liver disease	85 (42.5)
Uric acid (mg/dL) (mean ± S.D.)	5.97 ± 1.5
HOMA-IR (mean ± S.D.)	2.09 ± 0.6
HbA1c (mean ± S.D.)	6.02 ± 0.9

S.D., standard deviation; BMI, body mass index; HCV, hepatitis C virus; HBV, hepatitis B virus; HOMA-IR, Homeostatic Model Assessment of Insulin Resistance; HbA1c, haemoglobin A1C.
[a] Data are *n* (%) unless otherwise stated.

Table 2
Impact of treatment on Quality of Life in Reflux and Dyspepsia (QOLRAD) score, according to patient response to treatment.

	QOLRAD score (mean ± S.D)				*P*-value
	Total (*n* = 200)	Responders (*n* = 136)	Non-responders, FAT-positive (*n* = 15)	Non-responders, FAT-negative (*n* = 49)	
Baseline	82.4 ± 11.6	80.4 ± 14.2	84.7 ± 10.7	76.9 ± 12.6	0.08
After first-line therapy	97.2 ± 14.5	122.5 ± 20.6	87.3 ± 12.6	78.4 ± 9.6	0.001
After salvage therapy			112.6 ± 19.2	82.7 ± 13.3	0.023
After culture and AST			–	116.4 ± 10.6	

FAT, faecal antigen test; AST, antimicrobial susceptibility testing.

Table 3
Characterisation of study patients according to their response to treatment.[a]

	Responders (*n* = 136)	Non-responders, FAT-positive (*n* = 15)	Non-responders, FAT-negative (*n* = 49)	*P*-value
Male/female	86/50	9/6	39/10	0.01
Age (years) (mean ± S.D.)	35.3 ± 6.7	47.3 ± 5.6	45.2 ± 3.7	0.03
BMI (kg/m^2) (mean ± S.D.)	25.6 ± 1.8	25.3 ± 2.6	29.4 ± 2.7	0.01
Smoking	36 (26.5)	5 (33.3)	21 (42.9)	0.01
Diabetes mellitus	22 (16.2)	4 (26.7)	18 (36.7)	0.001
Previous macrolide exposure	10 (7.4)	10 (66.7)	28 (57.1)	0.001
HCV	13 (9.6)	5 (33.3)	20 (40.8)	0.001
HBV	7 (5.1)	1 (6.7)	5 (10.2)	0.06
Fatty liver disease				
No	96 (70.6)	7 (46.7)	12 (24.5)	0.001
Mild	29 (21.3)	1 (6.7)	8 (16.3)	0.001
Moderate	6 (4.4)	3 (20.0)	13 (26.5)	0.04
Severe	5 (3.7)	4 (26.7)	16 (32.7)	0.001
Uric acid (mg/dL) (mean ± S.D.)	5.6 ± 1.5	7.2 ± 1.1	6.4 ± 1.5	0.034
HOMA-IR (mean ± S.D.)	1.8 ± 0.4	2 ± 0.6	2.98 ± 0.3	0.01
HbA1c (mean ± S.D.)	5.5 ± 0.6	6.2 ± 0.8	7.3 ± 0.5	0.02
FAT (ng/mL) (mean ± S.D.)	10.2 ± 3.8	67.6 ± 16.3	23.3 ± 12.5	0.03

FAT, faecal antigen test; S.D., standard deviation; BMI, body mass index; HCV, hepatitis C virus; HBV, hepatitis B virus; HOMA-IR, Homeostatic Model Assessment of Insulin Resistance; HbA1c, haemoglobin A1C.
[a] Data are *n* (%) unless otherwise stated.

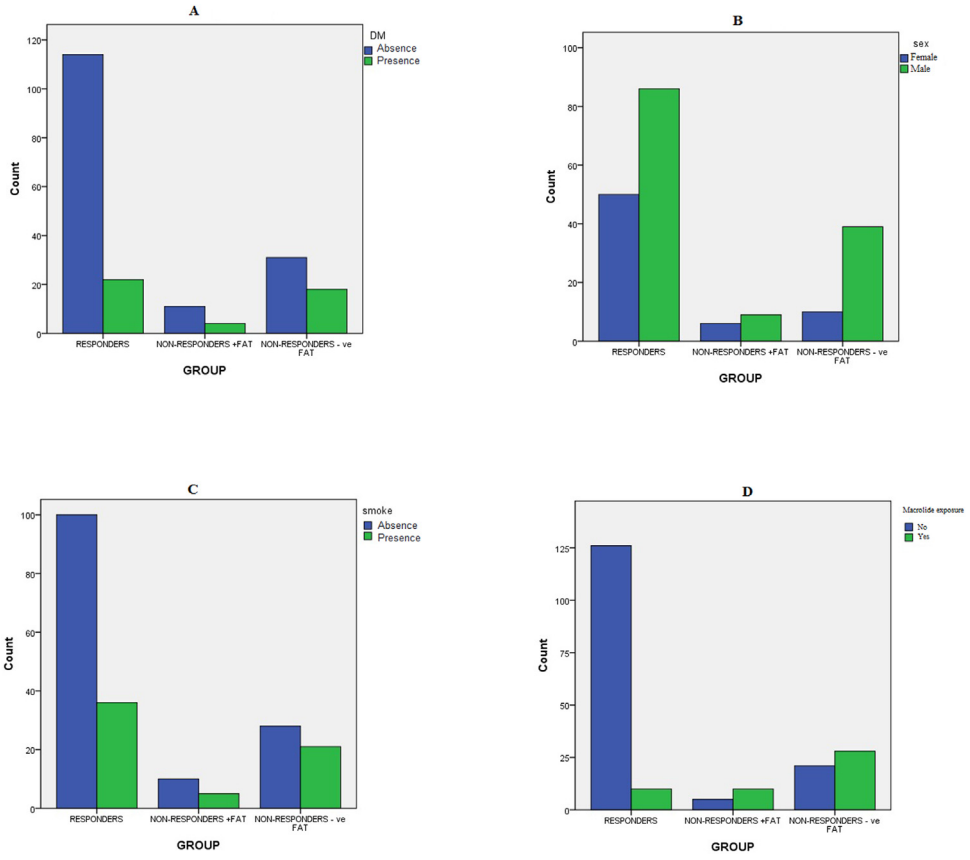

Fig. 2. (A) Prevalence of diabetes mellitus, (B) sex distribution, (C) prevalence of smoking and (D) previous macrolide exposure in the studied subgroups. FAT, faecal antigen test.

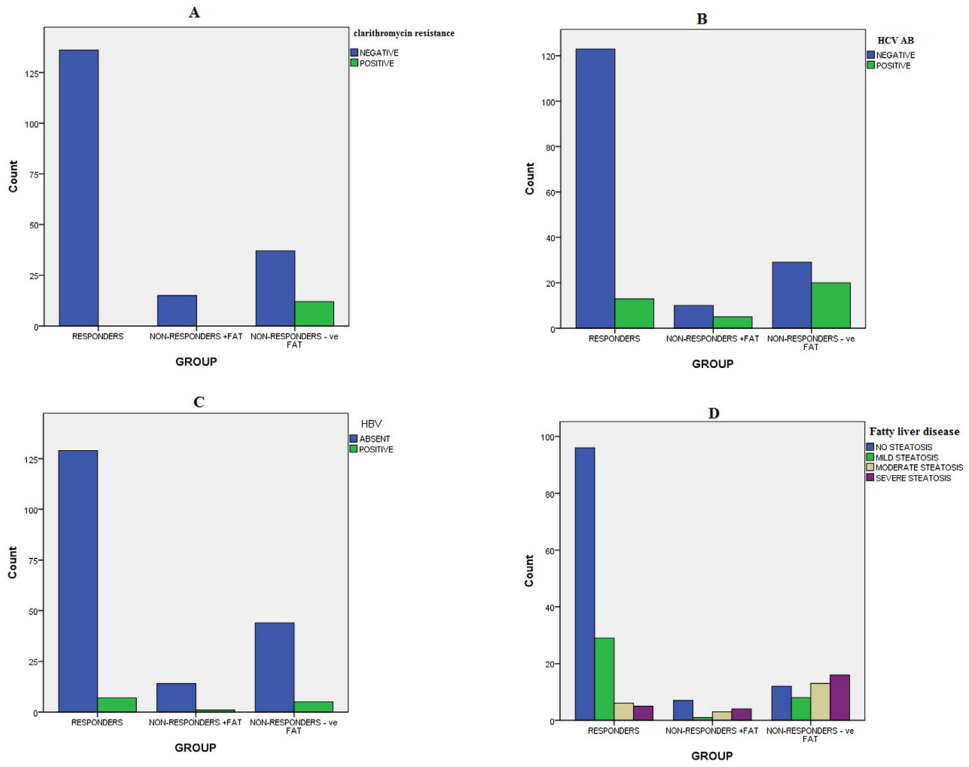

Fig. 3. Prevalence of (A) clarithromycin resistance, (B) hepatitis C virus (HCV), (C) hepatitis B virus (HBV) and (D) previous fatty liver disease in the studied subgroups. FAT, faecal antigen test.

Logistic regression analysis revealed that variables independently associated with persistence of *H. pylori* were HOMA-IR ($\beta = 5.49$, $P = 0.0001$) and severe fatty liver disease ($\beta = 6.1$, $P = 0.0001$).

4. Discussion

This study sheds light on a new form of chronic *Helicobacter*-induced gastritis (occult *Helicobacter* gastritis), which is defined as persistent symptoms despite negative FAT with a proven positivity of rapid urease test, which is highly sensitive and specific in the absence of active gastrointestinal bleeding [22]. Monoclonal *Helicobacter* faecal antigen is more accurate in detecting eradication of the infection, with sensitivity and specificity of 96% and 83%, respectively [23].

False-negative results of FAT and urea breath test could be encountered in case of a transient decrease in bacterial load induced by PPIs, antimicrobial agents, bleeding, premalignant conditions such as atrophic gastritis or intestinal metaplasia, and MALT lymphoma [24]. False-positive results may also occur in the case of gastric bacterial overgrowth with urease-producing bacteria other than *H. pylori* in acid-free stomach due to chronic use of PPIs [25].

Resistance to the standard clarithromycin-based triple therapy is currently increasing and this has a significant negative impact on eradication rates [26].

Mutations at the binding sites of macrolides, mainly A2143 G, A2142 G and A2142C in the 23S rRNA in domain V, would impair the binding of macrolides to the 50S ribosomal subunit [27].

In the current study, the prevalence of clarithromycin resistance in non-responders with negative FAT was 24.5%. However, a previous study on Malaysian patients showed a lower prevalence of 3% and this could be related to less frequent use of clarithromycin in their community [28].

There is scarce data discussing possible factors that predispose or enhance refractoriness of *H. pylori*. The current study revealed that BMI, age, HOMA-IR, previous macrolide exposure, severity of fatty liver disease and presence of HCV were highly correlated with persistence of *H. pylori*. Non-responders with negative FAT had moderate and severe fatty liver disease (26.5% and 32.7%, respectively).

The relationship between chronic *H. pylori* infection and fatty liver disease is via the presence of insulin resistance. The current study showed a positive correlation with insulin resistance, severity of fatty liver disease and persistence of *H. pylori*, as previously reported by Polyzos et al. [29]. *H. pylori* eradication has been associated with improvements in serum insulin levels and HOMA-IR and hence in insulin resistance [30].

HCV is associated with *H. pylori* infection in up to 56% of patients and this may be due to an affect on lymphocyte function [31,32]. In the current study, 40.9% of non-responders with negative FAT had chronic HCV.

In the current study, treatment of refractory *H. pylori* gastritis was evaluated by culture and AST, which revealed bacterial susceptibility to norfloxacin ($n = 18$ patients), moxifloxacin ($n = 13$), doxycycline ($n = 11$) and amikacin ($n = 8$), which were administered according to the results of the culture, together with nitazoxanide to gain the benefit of its potency against multidrug-resistant strains, thus boosting the therapeutic effects of culture-selected antimicrobial agents [33]; both were combined with high-dose esomeprazole [34].

A study in India evaluating norfloxacin at a dose of 400 mg twice daily with a PPI achieved an eradication rate of nearly 77% [35]. A significant percentage of the isolated *Helicobacter* strains were multidrug-resistant, and amikacin (30 μg) was among the antibiotics to which the organism was most susceptible [36]. A study conducted by Abd-Elsalam et al. revealed that 2-week nitazoxanide-based therapy is an effective and safe salvage therapy

in patients experiencing previous therapy failure [37]. Symptomatic improvement was achieved in 59.2% of patients despite confirmed eradication by rapid urease test.

Limitation of the current study are the fact that it was a single-centre study as well as the lack of motility studies for symptomatic patients following eradication of *H. pylori* by culture-based therapy, and this could be a point of future research.

In conclusion, refractory *H. pylori* gastritis is frequently seen in non-alcoholic fatty liver disease with insulin resistance and this requires gastric biopsy with culture and AST, together with review of the past history of antimicrobial use for the best selection and tailoring of eradication therapy in refractory and symptomatic patients.

Funding

None.

Competing interests

None declared.

Ethical approval

The study was approved by the Ethical Review Board of the Faculty of Medicine of Zagazig University, dated January 2016 [3270-16]. Written informed consent was obtained from each patient included in the study. The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in a priori approval by the institution's Human Research Committee.

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