

Helicobacter pylori and Systemic Disease

Guest Editors: Chao-Hung Kuo, Yen-Hsu Chen, Khean-Lee Goh,
and Lin-Li Chang





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Gastroenterology Research and Practice

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Editorial

Helicobacter pylori and Systemic Disease

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Currently, *Helicobacter pylori* (*H. pylori*) infection is confirmed to correlate with chronic gastritis, peptic ulcer disease, Mucosa Associated Lymphoid Tissue (MALT)-lymphoma, precancerous changes in the stomach (atrophy, intestinal metaplasia), and gastric cancer. At the same time, *H. pylori* eludes the immunological response evoked by the host. This chronic infection has the local production and systemic diffusion of proinflammatory cytokines, which may influence the remote organic systems and result in extragastric manifestations [1] (Table 1).

Several studies performed during the past years have supported the possible role for *H. pylori* infection in the pathogenesis of several extragastric diseases. The role of *H. pylori* in some hematologic conditions was included in the current guidelines, such as immune thrombocytopenic purpura (ITP), iron deficiency anemia (IDA), and vitamin B12 deficiency [2–4]. The effects on other systems such as cardiovascular diseases, diabetes mellitus, dermatological disease, and neurologic disorders have also attracted researchers' concern. Data known from those studies have shown that the immunological response caused by *H. pylori* might influence the clinical outcome of these diseases. However, many of these reports suffer from being case reports or case series without adequate controls.

The *H. pylori* eradication resulting in increasing the platelet count in adult patients with primary immune thrombocytopenia (ITP) has been confirmed [2, 4]. Moreover, there is sufficient evidence to regard *H. pylori* infection as a cause of unexplained sideropenic anemia (refractory IDA) by several mechanisms [3]. So, recent guidelines indicate *H. pylori*

infection to be sought in IDA patients. Other hematological diseases possibly related with *H. pylori* included monoclonal gammopathy, megaloblastic anemia, and myelodysplastic syndrome [5].

Many previous studies stated that chronic infection with *H. pylori* has significant interactions with the immune system. Recent epidemiological data suggest that aggressively eradicating *H. pylori* infection might be related to an increase in autoimmune diseases [6], but the possible mechanisms remain controversial. Many researchers thought that *H. pylori* have acquired several abilities that help them escape clearance through the host immune system. Then *H. pylori* interacts with the immune system and results in its downregulation. However, controversial results were reported in several studies. We need further research studies focusing on the possible impact of *H. pylori* on autoimmune diseases.

The relationship between seropositivity for *H. pylori* and outcome of cardiovascular disease is also an important issue. Previous studies have surveyed the association between *H. pylori* infection and coronary artery disease (CAD) [7]. The possible mechanisms of *H. pylori* infection in the pathogenesis of CAD include persistent local or systemic inflammation and initiating autoimmune responses [8]. However, the level of supporting evidence is too limited to advocate therapeutic interventions. Accordingly, further randomized trials are needed to evaluate the role of *H. pylori* eradication in these patients.

Some studies have disclosed that the association of lung cancer risk with *H. pylori* infection is five to ten times stronger than with passive smoking exposure [9]. It raises the notion

TABLE 1: The extragastric manifestation of *H. pylori* infection.

Involved extragastric system	Extragastric manifestations
Cardiovascular system	Atherosclerotic heart disease, cerebral vascular disease
Neurological system	Parkinson's disease, migraine
Hematological system	Immune thrombocytopenic purpura, iron deficiency anemia, Vit B12 deficiency anemia
Immunological system	Raynaud's phenomenon, Sjogren's syndrome
Dermatological system	Chronic urticaria, angioedema, alopecia areata
Endocrine system	Diabetes, autoimmune thyroiditis
Ear, nose, eye, and throat	Hyperemesis gravidarum, anorexia of aging, glaucoma, oral ulcers
Others	Halitosis, urethritis

that *H. pylori* might be a risk factor among non-smoking-related lung cancer. Many possible hypotheses have been proposed including the following: (a) the mechanisms may vary by both *H. pylori* strain and subtype of lung cancer; (b) *H. pylori* infection status/eradication should influence the clinical outcome of lung cancer; and (c) this association should be influenced by other factors [10]. However, the possible mechanisms and evidence need more studies to confirm any of these.

The role of *H. pylori* in dermatological diseases is still a controversial subject. The association between chronic urticaria (CU) and *H. pylori* has been found by some research groups [11]. The evidence comes from studies demonstrating that many patients with CU received clinical improvement after *H. pylori* eradication [12]. But recent trials, utilizing the Grading of Recommendations Assessment, Development, and Evaluation (GRADE) approach, showed different results where the benefit of *H. pylori* eradication in patients with CU was weak [13]. Other skin diseases also show controversial results and need further survey.

One recent meta-analysis stated that Type 2 diabetes and insulin use in diabetic patients are significantly associated with a higher incidence of *H. pylori* eradication [14]. Previous studies revealed that higher serological positivity of *H. pylori* were noted in patients with type 1 diabetes (T1DM) and autoimmune thyroiditis (AT). In their results, *H. pylori* infection could be considered as an environmental trigger for development of AT in T1DM. They suggested that young patients with T1DM should be screened for *H. pylori* infection [15].

When discussing medical economic policy, the relationship between *H. pylori* and systemic disease needs more attention. The population-based strategies for *H. pylori* eradication in people with low prevalence are unlikely to be cost-effective, but this management might be necessary in people with high risk of developing systemic disease. The challenge we face is to investigate whether, at what magnitude, and in which direction, *H. pylori* may be linked to systemic diseases and in which populations. We hope that this special issue will be helpful in the possible pathogenesis of *H. pylori* related extragastric manifestations.

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Research Article

Performance of Routine *Helicobacter pylori* Invasive Tests in Patients with Dyspepsia

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Background. This study was designed to compare the accuracy of three different invasive methods for the detection of *Helicobacter pylori* (*H. pylori*) infection in patients with dyspepsia. These tests included culture, histology, and the rapid urease test (CLO test). **Methods.** *H. pylori* infection was diagnosed prospectively in 246 untreated dyspeptic patients who underwent upper gastrointestinal endoscopy. The gold standard for *H. pylori* infection was based on a positive culture or both a positive histological examination and a CLO test. **Results.** *H. pylori* was diagnosed in 33.3% of the patients. The sensitivity, specificity, positive predictive value, negative predictive value, and overall accuracy were as follows: histology from the antrum (95.12; 95.12; 90.7; 97.5; 95.12%); histology from the antrum and corpus (95.12; 95.12; 90.7; 97.5; 95.12%); histology from the corpus (76.83; 96.95; 92.65; 89.33; 90.24%); culture (91.46; 100; 100; 95.91; 97.15%); a CLO test from the antrum and corpus (85.59; 100; 100; 93.71; 95.52%); a CLO test from the antrum (64.63; 100; 100; 84.97; 88.21%); a CLO test from the corpus (69.51; 100; 100; 96.77; 89.83%), respectively. **Conclusions.** Antral biopsy histology and culture are the best methods for the diagnosis of *H. pylori* infection in our cohort of patients with dyspepsia.

1. Introduction

Helicobacter pylori infection is very common worldwide, occurring in 40% to 50% of the population in developed countries, in 80% to 90% of the population in developing regions [1], and about 50% of the population in Taiwan [2]. The infection causes chronic gastritis which significantly increases the risk of developing gastric or duodenal ulcer [3, 4], gastric adenocarcinoma, and mucosa-associated lymphoid tissue (MALT) lymphoma [5, 6]. As the eradication of *H. pylori* has been shown to improve the outcome of peptic ulcer disease in terms of recurrence and complications, the accurate diagnosis of *H. pylori* infection is of clinical importance.

Several methods have been developed for the detection of *H. pylori* infection. These methods include noninvasive

tests that are based on the detection of antibodies to *H. pylori* or the urea breath test (UBT) or invasive tests that require endoscopy to obtain tissue biopsies, such as the rapid urease test (RUT), culture, and histological examination [7–9]. Each test has advantages and disadvantages, which make it more or less appropriate for different situations. Of all the available tests, invasive tests are considered the most accurate. However, invasive tests are mainly limited by their proneness to sampling error, because of the patchy distribution of the bacteria throughout the stomach [10, 11]. These circumstances yield the possibility of false negative results if the biopsy is taken from the antrum or the corpus alone. Studies on biopsy sites for the diagnosis of *H. pylori* infection are sometimes conflicting. Antrum biopsy is recommended by Genta and Graham [12], while others recommend at least one corpus

biopsy [13, 14]. So far no optimal biopsy site for the diagnosis of *H. pylori* status has been currently established.

This study has been designed and undertaken to compare the sensitivity, specificity, and accuracy of different invasive tests and biopsy sites for the diagnosis of *H. pylori* infection in clinical practice.

2. Methods

Patients with dyspepsia undergoing upper gastrointestinal endoscopy at Taipei City Hospital Ren-Ai Branch, Taipei, Taiwan, between March 2013 and July 2013, were included in this study. According to the Rome III criteria, dyspepsia is defined as one or more of the following symptoms: postprandial fullness, early satiation, and epigastric pain or burning [15]. Exclusion criteria were the following: bismuth salts, proton pump inhibitors, or antibiotic therapy within the last 2 months, previous *H. pylori* eradication therapy, chronic use of corticosteroids or immunosuppressants, prior gastric surgery, the presence of a bleeding peptic ulcer, severe concomitant disease, and pregnancy or lactation. All patients were informed of the objective of the study and subsequently gave informed consent in writing. This study was approved by the Ethics Committee of the Taipei City Hospital.

During endoscopic examination, several biopsy specimens were taken from each patient for histological examination: two from the antrum, one from the incisura angularis, and one from the corpus. For the rapid urease test, one was taken from the antrum and one from the body. For culture, one specimen was taken from the antrum.

2.1. Diagnostic Methods

2.1.1. Histology. Biopsy specimens were fixed in formalin and sections were assessed for the presence of *H. pylori* by a modified Giemsa stain. The degree of inflammatory cell infiltration, atrophy, and intestinal metaplasia was assessed in sections stained with hematoxylin and eosin (H&E). The histological features of the antrum and body of the gastric mucosa were graded according to the updated Sydney System.

Histology (antrum) included two biopsy specimens from the antrum and one from the incisura angularis (the lesser curvature). Histological examination of two biopsy specimens from the corpus was also carried out.

2.1.2. Culture. One antrum biopsy specimen from each patient was cultured. The specimen was rubbed across the surface of a CampyBAP agar plate (Brucella agar (Difco) + IsoVitaleX (Gibco) + 10% whole sheep's blood), and the plate was incubated at 35°C under microaerobic conditions (5% O₂, 10% CO₂, and 85% N₂) for 4-5 days. The culture was considered to be *H. pylori* positive if one or more colonies of spiral or curved Gram-negative, oxidase (+), catalase (+), and urease (+) rods were present.

2.1.3. Rapid Urease Test. Two biopsy specimens, one from the antrum and one from the corpus, were tested from each patient. The specimens were subjected to a rapid urease test

TABLE 1: Demographic characteristics of patients.

Age-year, mean (range) y/o	53.1 (22–90)	
Gender-number		
Male	106	43.1%
Female	140	56.9%
<i>H. pylori</i> status		
Infection	82	33.3%
Noninfection	164	66.7%
Peptic ulcer-number		
Gastric ulcer	77	31.3%
Duodenal ulcer	35	14.2%
Antrum atrophy		
Normal to mild	244	99.2%
Moderate to severe	2	0.8%
Corpus atrophy		
Normal to mild	237	96.3%
Moderate to severe	9	3.7%
Antrum intestinal metaplasia		
Normal to mild	221	89.8%
Moderate to severe	25	10.2%
Corpus intestinal metaplasia		
Normal to mild	242	98.4%
Moderate to severe	4	1.6%

TABLE 2: Results of detection of *H. pylori* by each test.

Test	Positive	Negative
Culture	75 (30.4%)	171 (69.6%)
Histology (antrum)	86 (35.0%)	160 (65.0%)
Histology (corpus)	68 (27.6%)	178 (73.4%)
Histology (antrum and corpus)	89 (36.2%)	157 (63.8%)
CLO test (antrum)	53 (21.5%)	193 (78.5%)
CLO test (corpus)	57 (23.2%)	189 (76.8%)
CLO test (antrum and corpus)	71 (28.9%)	175 (71.1%)

(CLO test, Kimberly-Clark, USA), to detect the presence of *H. pylori* urease. A positive result was reported if the color changed from yellow to pink within 24 h of incubation at room temperature.

2.1.4. Gold Standard Definition. A patient was defined as being *H. pylori* positive on the basis of a positive culture or, in the case of a negative culture, both a positive histological examination and a positive rapid urease test (CLO test).

2.1.5. Statistical Analysis. Standard methods were used to calculate the sensitivity, specificity, positive and negative predictive values, and accuracy. All statistical analyses, data collection, and manipulation were carried out using the Statistical Package for Social Sciences (SPSS 19.0 for Windows, SPSS Inc., Chicago, IL, USA).

TABLE 3: Results of the detection of *H. pylori* by each test according to the “gold standard” definition.

Test	Result	<i>H. pylori</i> positive (n)	<i>H. pylori</i> negative (n)
Culture	Positive	75	0
	Negative	7	164
Histology (antrum)	Positive	78	8
	Negative	4	156
Histology (corpus)	Positive	63	5
	Negative	19	159
Histology (antrum and corpus)	Positive	78	8
	Negative	4	156
CLO test (antrum)	Positive	53	0
	Negative	29	164
CLO test (corpus)	Positive	57	0
	Negative	25	164
CLO test (antrum and corpus)	Positive	71	0
	Negative	11	164

TABLE 4: Results of the sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy among the tests.

Test	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)
Culture	91.46	100	100	95.91	97.15
Histology (antrum)	95.12	95.12	90.70	97.50	95.12
Histology (corpus)	76.83	96.95	92.65	89.33	90.24
Histology (antrum and corpus)	95.12	95.12	90.70	97.50	95.12
CLO test (antrum)	64.63	100	100	84.97	88.21
CLO test (corpus)	69.51	100	100	96.77	89.83
CLO test (antrum and corpus)	86.59	100	100	93.71	95.52

3. Results

A total of 246 patients were enrolled in this study, 106 (43.1%) were male and 140 (56.9%) were female. The age of the subjects ranged from 22 to 90 years, the mean being 53.1 ± 15.0 years. Patient characteristics, including the number infected by *H. pylori*, the number with peptic ulcers, atrophy, and intestinal metaplasia, are shown in Table 1. According to the golden standard definition, the overall *H. pylori* infection rate was 33.3%.

Table 2 shows the results of the diagnosis of *H. pylori* infection by each method in this study. Of the 246 patients, culture was positive in 75 patients (30.4%) and negative in 171 (69.6%). The CLO test (antrum and corpus) was positive in 71 patients (28.9%) and negative in 175 (71.1%). Histology (antrum and corpus) was positive in 89 patients (36.2%) and negative in 157 (63.8%).

The status of *H. pylori* infection, according to the gold standard criteria, is shown in Table 3. The sensitivity, specificity, accuracy, positive predictive value (PPV), and negative predictive value (NPV) of each diagnostic test were calculated and results are shown in Table 4. The sensitivity was good for culture (91.46%), histology (antrum), and histology (antrum and corpus) (95.12% each), but only 64.63% for the CLO test (antrum) and 69.51% for CLO test (corpus). The specificity was the best for culture, CLO test (antrum and corpus), CLO

test (antrum), CLO test (corpus) (100% each), followed by histology (corpus) (96.95%), histology (antrum), and histology (antrum and corpus) (95.12% each).

The overall accuracy was equally good in culture (97.15%), followed by the CLO test (antrum and corpus) (95.52%), histology (antrum) and histology (antrum and corpus) (95.12% each), with the poorest overall accuracy being for the CLO test (antrum) (88.21%).

4. Discussion

The use of a single test as the gold standard increases the error rate [16] and it is therefore recommended that the gold standard determination of infection should depend on results from two tests [17]. The gold standard in our study is a positive culture. However, if the culture is negative, positive histology and positive urease test combined correspond to acceptable criteria.

Although all the biopsies from invasive tests may give false negatives in the case of low density and patchy distribution of *H. pylori*, both histology and culture were among the best-performing tests in our study. The sensitivity of *H. pylori* culture is an important issue, and the method is not routinely recommended as the primary tool for identifying the presence of the infection [18]. However, it is becoming increasingly important in populations with high antibacterial

resistance [19]. We found 91.4% sensitivity, 100% specificity, and 97.15% accuracy with this method.

Histology is a highly sensitive method for determining *H. pylori* infection and also provides insight into the status of the gastric mucosa. However, the disadvantages of this technique depend not only on the quality of the biopsy specimens, but also the expertise of the pathologists [20]. Our study demonstrated that two biopsy specimens from the antrum and one biopsy from the incisura angularis provided high diagnostic sensitivity and specificity, with an accuracy greater than 90%. But histological examination of two corpus biopsy specimens showed low sensitivity. Sampling errors, insufficient bacterial load [21], bacterial clearance [13], and patchy bacterial distribution are common causes of false negative results in corpus biopsy histology. Furthermore, biopsy specimens from both antrum and corpus showed the same sensitivity as compared with an antrum biopsy in dyspeptic patients. This is because when corpus *H. pylori* infection occurs, antrum *H. pylori* infection exists already. Our studies have identified high antral biopsy histology performance.

In the presence of *H. pylori* urease, urea is hydrolyzed, leading to a rise in pH and color change of the pH indicator (phenol red). The CLO test has been established for more than a decade and used as the main standard for rapid urease tests. The test has good performance, with a sensitivity of 89.6%, a specificity of 100%, a PPV of 100%, and a NPV of 84.1%, and has been widely acclaimed [22]. Our data showed similar results when one antrum biopsy and one corpus biopsy were combined. But a single antrum biopsy or corpus biopsy failed to meet the expectations and demonstrated low sensitivity (64.63% and 69.51%). Some previous studies have shown false negative results in up to 50% of patients over 60 years of age [23]. Mégraud had also reported low CLO test sensitivity [24]. Woo et al. reported that increasing the number of biopsies to more than one or adding extra biopsy sites may increase the sensitivity since this probably increases the *H. pylori* load and consequently the amount of urease [25]. However, this prolongs endoscopy time and adds to the discomfort of the patient.

The major limitation of this study is a lack of global evaluation tests, such as the urea breath test or the stool *H. pylori* antigen test, which have been found reliable for the detection of the presence of *H. pylori*. Sampling bias may exist within our biopsy-based diagnostic tests, including culture, histology, and the CLO test. Furthermore, the prevalence of *H. pylori* infection was low in our study cohort. It may be that our patients were mainly from an urban area (Taipei City) and might have had a lower *H. pylori* infection rate [2].

5. Conclusion

Antral biopsy histology and culture are the best methods for the diagnosis of *H. pylori* infection in our cohort of patients with dyspepsia. The low sensitivity of corpus biopsy histology, and of the antral or corpus biopsy CLO test, makes these seem inappropriate for the determination of the *H. pylori* status in our population.

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Research Article

Effect of Di(2-ethylhexyl)phthalate on *Helicobacter pylori*-Induced Apoptosis in AGS Cells

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Plastic products are widely used in human life. Di(2-ethylhexyl)phthalate (DEHP) is an essential additive in plastic manufacturing and is used as plasticizer for many products including plastic food packaging. DEHP is a teratogenic compound and can cause potent reproductive toxicity. DEHP can also cause liver damage, peroxisome proliferation, and carcinogenesis. DEHP is also strongly associated with peptic ulcers and gastric cancer; however, the underlying effect and mechanism of DEHP on the gastrointestinal tract are not entirely clear. The oral infection route of *H. pylori* parallels the major ingestion route of DEHP into the human body. Therefore, we wanted to study the effect of DEHP and *H. pylori* exposure on the human gastric epithelial cell line, AGS (gastric adenocarcinoma). The viability of the AGS cell line was significantly lower in 80 μ M-DEHP and *H. pylori* (MOI = 100 : 1) coexposure than DEHP or *H. pylori* alone. DEHP and *H. pylori* coexposure also induced caspase-3 activation, and increased Bax/Bcl-2 ratio and DNA fragmentation in AGS cells. These results indicate that DEHP can enhance *H. pylori* cytotoxicity and induce gastric epithelial cell apoptosis. Therefore, it is possible that DEHP and *H. pylori* coexposure might enhance the disruption of the gastric mucosa integrity and potentially promote the pathogenesis of gastric carcinogenesis.

1. Introduction

Di(2-ethylhexyl)phthalate (DEHP) is the most common plasticizer used to increase the flexibility of polyvinyl chloride (PVC). DEHP is often used for the development of flexible plastics in food-packaging, plastic flooring, carpet material,

roofing materials, plastic wall treatments, indoor decorations, wire, cable packaging materials, and children's toys [1]. DEHP is also used as a cleaner, industrial solvent, wetting agent, and lubricant [2]. Human exposure to DEHP usually occurs through air, water, or skin contact with DEHP-contained plastics [3]; however, the most common source of exposure

is due to the ingestion of contaminated packaged foods. Due to its chemical structure, DEHP can readily dissolve and seep from the packaging materials into the food [2–4].

Phthalate exposure can have many potential health effects in humans. For example, a previous study reported that DEHP and mono-2-ethylhexyl phthalate (MEHP) can pass through the placenta and shorten the gestational period of a developing fetus [5]. DEHP also delays the development of the male reproductive system [2]. Recent evidences also show prenatal DEHP exposure is associated with shorter gestation [6], but prenatal DEHP exposure does not affect birth outcomes [7]. Even though oral ingestion of DEHP is one of the most common routes of exposure in humans, the effects of this toxin on gastric epithelial cells have not been fully elucidated.

The human gastric pathogen, *Helicobacter pylori* (*H. pylori*), is a spiral Gram-negative microaerophilic bacterium, which can selectively colonize the mucus layer of the stomach and can cause severe gastric problems including the development of chronic gastritis, peptic ulcers, and gastric cancer [8]. *H. pylori* is often transmitted to human through a variety of ways, including oral-oral and fecal-oral routes [9]. *H. pylori* infection induces apoptosis, of gastric epithelial cells, an effect which was reported with both in vivo [10, 11] and in vitro studies [12–14].

Plastics are widely used in food packaging in Taiwan and the world; however, DEHP exposure is often higher in the Taiwanese population than other countries such as in Germany or the US [15, 16]. *H. pylori* infection occurs in approximately 50% of the world's population. The Taiwanese population has as a 54.4% antibody seropositivity against *H. pylori* [17], which is higher than the *H. pylori* seropositivity in other countries, such as Ireland [18]. Taken together, these data demonstrate that the high exposure rate of DEHP and high infection rate of *H. pylori* in the Taiwanese people, as well as with the rest of the world, are likely an important health concern; however, the effect of DEHP and *H. pylori* coexposure on gastric epithelial cells is not well understood. For this reason, the effect of DEHP and *H. pylori* coexposure on gastric epithelial cell apoptosis, as an indicator of reduced epithelial cell integrity, was investigated in this study.

2. Materials and Methods

2.1. Cell Culture. A human gastric epithelial cell line, AGS (gastric adenocarcinoma, BCRC 60102), was purchased from the Cell Bank of the Taiwan National Health Research Institute and was grown in RPMI 1640 medium supplemented with 10% fetal bovine serum, 100 U/mL penicillin, and 100 µg/mL streptomycin or 0.1% gentamicin (Hyclone, Logan, UT, USA). AGS cells were seeded at densities of $2\sim 2.5 \times 10^5$ /35-mm culture dish or $5\sim 5.5 \times 10^5$ /25 T flask, incubated for 24 hr, and switched to culture medium containing 0~80 µM DEHP (CAS number: 117-81-7, Sigma-Aldrich, St. Louis, MO, USA). DEHP has limited solubility in water; a stock solution of DEHP was prepared in DMSO and subsequently diluted to various concentrations with cell culture medium. The final concentration of DMSO in culture medium was approximately 1% and had no significant effect

on cell viability. All cultures were grown in a humidified incubator at 37°C and in an atmosphere of 95% air-5% CO₂.

2.2. *H. pylori* Culture. Due to the role of cytotoxin associated gene A (CagA) and vacuolating cytotoxin A (VacA) genes, genes commonly associated with *H. pylori* associated gastric cell apoptosis [19], the CagA/VacA positive *H. pylori* strain ATCC 43054, purchased from the Cell Bank of the Taiwan National Health Research Institute, was used for this study. ATCC 43054 was cultured on trypticase soy agar with 5% sheep's blood (Curtin Matheson, Jessup, MD, USA) with Skirrow's selective antibiotic supplement (Prolab Inc., Scarborough, Canada) at 37°C in a CO₂/O₂ water jacketed incubator (Forma Scientific, Marietta, OH, USA) under microaerophilic conditions (10% CO₂, 7.5% O₂, 82.5% N₂). *H. pylori* were added to cells at a bacterium: cellular concentration range (Multiplicities of infection, MOI) of 100 : 1 to 25 : 1. *H. pylori* was used between passages 5 and 15 for these experiments to ensure that the bacteria were able to readily adhere to AGS cells. Adherence was visualized using microscopy.

2.3. Cell Viability Assay. AGS cells were seeded in 48-well culture plates at a density of 100 cells/mm², allowed to grow for 24 hr, and switched to culture media containing DEHP with or without *H. pylori* for 18 to 48 hr. For measuring the cell viability, MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide, 0.5 mg/mL; Sigma-Aldrich, St. Louis, MO, USA) was added to each well, and the plates were incubated for 3 hours at 37°C. The formazan crystals (a product of metabolic activity) were dissolved in isopropyl alcohol with 0.04% HCl and the formazan in each well was quantified using a Dynex MRX II spectrophotometer (Dynex Technologies, Chantilly, VA, USA) at absorption frequencies of 540 and 630 nm. The data were pooled from three independent experiments at least, and the number of replicate wells is ≥4.

2.4. DNA Fragmentation Assay. The extent of DNA fragmentation was quantified using the Cell Death Detection ELISA^{plus} kit (Roche, Mannheim, Germany) as described in the manufacturer's manual. Briefly, cells were lysed by adding lysis buffer to each well and incubating for 30 min at 4°C. Each plate was centrifuged at 200 ×g for 10 min, and 20 µL of each supernatant was transferred to streptavidin-coated wells. The wells were treated with an anti-histone- and anti-DNA-containing immuno-reagent, incubated for 2 hr at room temperature, washed three times, and treated with the peroxidase substrate 2,2'-azino-di-(3-ethyl-benzthiazoline sulfonate). Absorption at 405 nm was measured using a Dynex MRX II spectrophotometer (Dynex Technologies, Billingshurst, UK).

2.5. Immunoblotting Analysis of Caspase-3, Caspase-8, Bax, and Bcl-2. Cell protein extracts (20 µg) were denatured in sodium dodecyl sulfate (SDS) sample buffer at 95°C for 5 min, loaded onto 10–20% gradient SDS-polyacrylamide gel electrophoresis (PAGE) gels (Invitrogen), and separated by electrophoresis. Separated proteins were transferred to a polyvinylidene difluoride (PVDF) membrane. The membrane was blocked with 5% nonfat milk in Tris-buffered saline

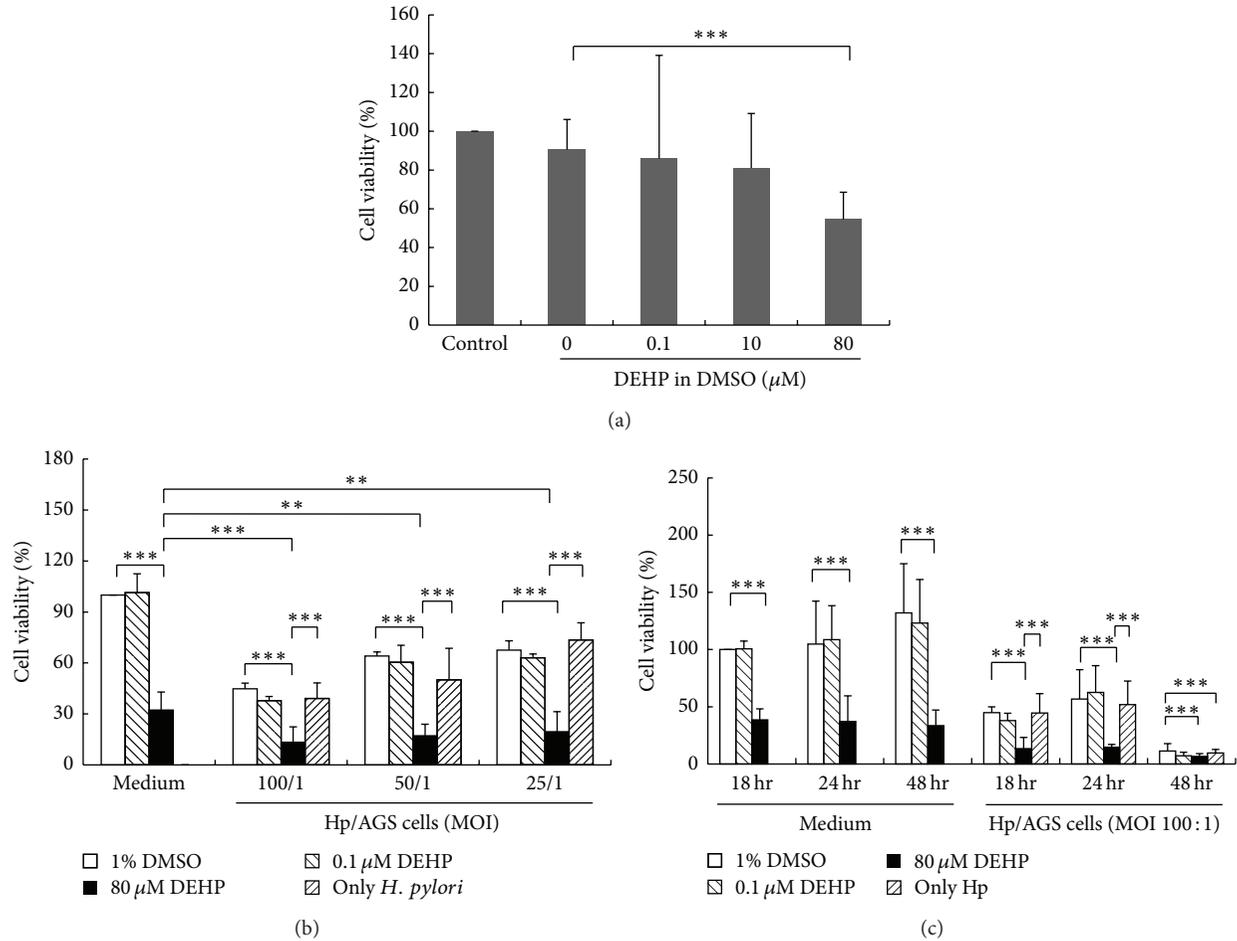


FIGURE 1: The cell viability of AGS cell. (a) Cells were treated with 0.1 to 80 μM DEHP for 24 hours. (b) Cells were treated 0.1 and 80 μM DEHP alone or combined with *H. pylori* (MOI = 100 : 1~25 : 1) for 18 hours (ten independent experiments). (c) Cells were treated 0.1 and 80 μM DEHP alone or combined with *H. pylori* (MOI = 100 : 1) for 18, 24, and 48 hours. Values obtained from MTT assay in mean + SD and normalized to nontreated value ($N = 7$, three independent experiments, $**P < 0.05$, $***P < 0.01$, one way ANOVA assay with LSD *post hoc* test).

(TBS) containing 0.05% Tween-20 and treated with antibodies against active caspase-3 (rabbit polyclonal antiactive caspase-3 antibody, 1:500, Abcam, Cambridge, MA, USA), active caspase-8 (rabbit polyclonal antiactive caspase-8 antibody, 1:1000, Abcam,), Bax (mouse polyclonal anti-Bax antibody, 1:500, Santa Cruz Biotechnology (SCBT), Santa Cruz, CA, USA), Bcl-2 (mouse polyclonal anti-Bcl-2 antibody, 1:500, SCBT), GAPDH (chicken polyclonal anti-GAPDH antibody, 1:1000, Millipore, Billerica, MA, USA) and β -actin (mouse monoclonal anti- β -actin antibody, 1:1000, SCBT). Bound antibodies were detected using HRP-conjugated secondary antibodies (1:5000, Jackson ImmunoResearch Laboratories; West Grove, PA, USA) and enhanced chemiluminescence (ECL, Amersham Pharmacia Biotech, Piscataway, NJ, USA). Densitometric images of blots were visualized on a BioSpectrum AC Imaging System (UVP, Upland, CA, USA) and were analyzed using Vision WorksLS analysis software.

2.6. Statistical Analysis. At least triplicate experiments were performed for each set of operating conditions. The quantitative data are expressed as mean \pm SD. Statistics were performed using SigmaStat 3.5 (SysStat Software, San Jose, CA, USA). Differences between study and control groups were evaluated by analysis of variance (ANOVA). The level of significance for differences between groups was further analyzed using post hoc Fisher's least significant difference (LSD) tests. A $P < 0.05$ was defined as statistically significant.

3. Result

3.1. DEHP and *H. pylori* Coexposure Reduced Cell Viability of AGS Cells. The cell viability of AGS cells after DEHP treatment for 24 hr is shown in Figure 1(a). Cell viability was significantly decreased after an exposure of 80 μM DEHP treatment (one way ANOVA, $P < 0.001$). The 0.1 μM ($P = 0.486$), 1 μM ($P = 0.215$), and 10 μM ($P = 0.375$)

of DEHP also induced a nonsignificant decrease in AGS cell viability, when compared with a DMSO (vehicle) group and tested by one way ANOVA. Additionally, no significant statistical difference (one way ANOVA, $P = 0.427$) was observed between control (culture medium without DMSO) and vehicle group. Since the DEHP-80 μM caused the most significant decrease in cell viability, this concentration was utilized for further experiments.

AGS cell viability after DEHP and *H. pylori* coexposure are presented in Figure 1(b). Cell viability was significantly reduced by DEHP-80 μM for 18 hr (one way ANOVA, $P < 0.01$). *H. pylori* infection significantly increased AGS cell death in a (multiplicity of infection) MOI-dependent manner. Compared with vehicle or *H. pylori* alone, DEHP-80 μM and *H. pylori* coexposure significantly reduced the cell viability of AGS cell after an 18 hr exposure (one way ANOVA, $P < 0.01$). Compared with DEHP-80 μM alone, DEHP-80 μM and *H. pylori* coexposure also significantly reduced the cell viability of AGS cell after *H. pylori* (MOI = 100/1) coexposure (one way ANOVA, $P < 0.01$). Not only does this result imply a cytotoxic effect of DEHP and *H. pylori* alone on AGS cells, but it also indicates that the combined DEHP and *H. pylori* exposure has an additive cytotoxic effect on AGS cells. Since the cytotoxicity of *H. pylori* at a MOI of 100/1 when combined with DEHP was higher than at MOI of 50/1 and MOI of 25/1, this experimental condition was used for further studies. In addition, there was no significant statistical difference in cell viability between the groups of *H. pylori* in DMSO (vehicle) and *H. pylori* (MOI = 100:1, 50:1 and 25:1) in culture medium (one way ANOVA, $P = 0.613$, $P = 0.149$, and $P = 0.452$ resp.).

A time-course study of AGS cell viability changes after DEHP and *H. pylori* coexposure are shown in Figure 1(c). The cell viability was significantly decreased in a time-dependent manner by DEHP-80 μM treatment (one way ANOVA, $P < 0.01$). DEHP-80 μM and *H. pylori* (MOI = 100:1) coexposure more significantly decreased AGS cell viability. Because almost cells (about 87%) were dead after 18 hr of exposure and cell viability was not significantly statistical difference between 18 hr and 24 hr after DEHP-80 μM and *H. pylori* coexposure (one way ANOVA, $P = 0.674$), therefore, DEHP and *H. pylori* treatment for 18 hr was selected for further experiments.

3.2. DEHP and *H. pylori* Coexposure-Induced Apoptosis of AGS Cells. The Cell Death Detection^{Plus} system (Roche) was used to detect AGS cell apoptosis (Figure 2). DEHP dose dependently induced a nonsignificant increase in the ratio of DNA fragmentation. Compared with a vehicle group, an 18 hr exposure of DEHP-80 μM exhibited a nonsignificant tendency to increase the DNA fragmentation ratio (one-way ANOVA, $P = 0.059$). DEHP and *H. pylori* (MOI of 100/1) coexposure for 18 hr, however, significantly increased the DNA fragmentation ratio (one-way ANOVA, $P < 0.01$). The ratio of DNA fragmentation after DEHP-80 μM and *H. pylori* coexposure was significantly higher than DEHP (one-way ANOVA, $P < 0.01$) or *H. pylori* alone (one-way ANOVA, $P < 0.01$). This result implies that DEHP can enhance the toxicity of *H. pylori* and increase *H. pylori*-induced apoptosis

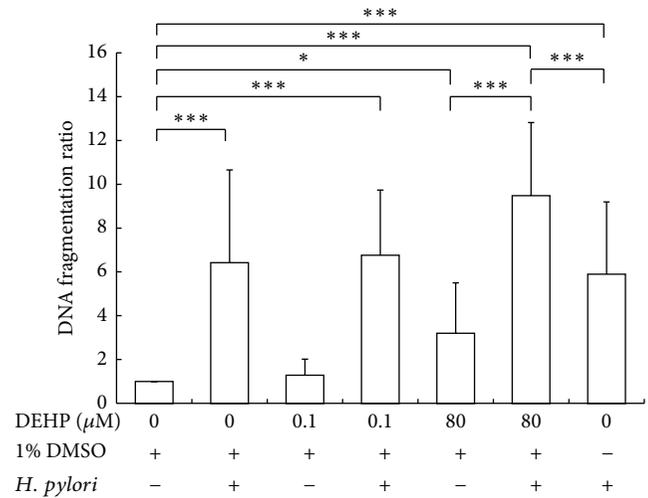


FIGURE 2: DEHP and *H. pylori* (MOI = 100:1) exposure induced DNA fragmentation of AGS cells. The level of apoptosis occurring with each treatment was determined by cell death ELISA^{Plus} kit. Statistical significance was analyzed by one-way ANOVA and post hoc LSD test (* $P < 0.1$, ** $P < 0.05$, *** $P < 0.01$, $N = 4$).

of AGS cells. Additionally, the ratio of *H. pylori*-induced DNA fragmentation did not exhibit a significant difference in DMSO-containing or normal medium, which indicates the activity and toxicity of *H. pylori* were not affected by DMSO.

3.3. DEHP and *H. pylori* Coexposure Increased Bax/Bcl-2 Ratio. Expression of Bax and Bcl-2 protein after DEHP and *H. pylori* treatment for 3 hours was detected by Western blot analysis and the Bax/Bcl-2 ratio was calculated. DEHP-80 μM (one way ANOVA, $P < 0.05$) or *H. pylori* treatment alone (one way ANOVA, $P < 0.05$) increase Bax/Bcl-2 ratio; however, the combined treatment of DEHP-80 μM and *H. pylori* coexposure caused a much higher Bax/Bcl-2 ratio increase (one way ANOVA, $P < 0.05$) (Figure 3). This result implied that DEHP and *H. pylori* coexposure disturbed the balance between Bax and Bcl-2 protein expression.

3.4. Effect of DEHP and *H. pylori* Coexposure on Expression of the Active Forms of Caspase-3 and Caspase-8. Expression of active caspase-3 and caspase-8 protein after 18 hr DEHP and *H. pylori* treatment was quantified by Western blot analysis and the results are presented in Figures 4 and 5. Expression of active caspase-3 was significantly increased after *H. pylori*/DEHP co-exposure, especially with DEHP-80 μM combined with *H. pylori* treatment (one way ANOVA, $P < 0.01$, compared with vehicle group) (Figure 4). DEHP-80 μM /*H. pylori* co-exposure-induced active caspase-3 protein expression, which was also higher than DEHP-80 μM (one way ANOVA, $P < 0.05$) or *H. pylori* alone (one way ANOVA, $P < 0.05$). Active caspase-8 expression was not significantly changed after 18 hr DEHP or *H. pylori* (one way ANOVA, $P > 0.1$) (Figure 5). The ratio of these apoptosis-related proteins also was summarized in Table 1 with mean \pm SD.

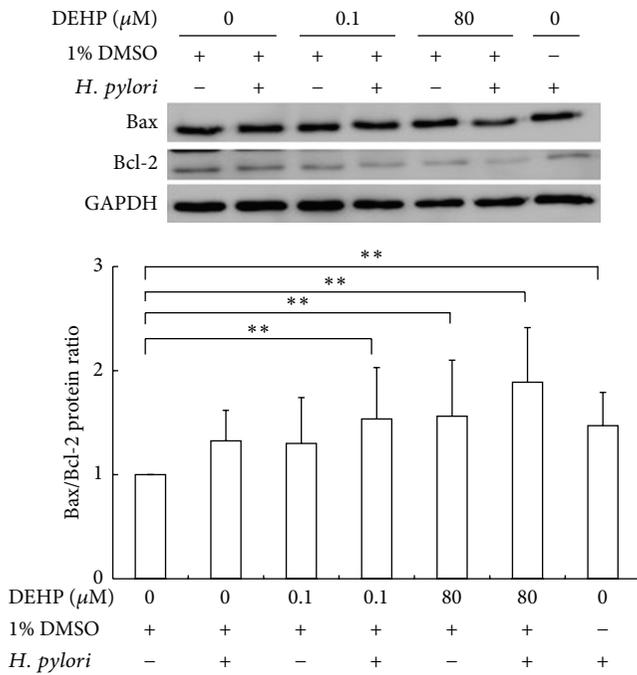


FIGURE 3: DEHP and *H. pylori* exposure for 3 hours changed the Bax/Bcl-2 ratio of AGS cells. Statistical significance was analyzed by one-way ANOVA and post hoc LSD test (** $P < 0.05$, $N = 4$).

4. Discussion

Helicobacter pylori (*H. pylori*) bacterium was first identified and isolated from gastric biopsies of patients with gastritis and peptic ulcers [20]. The NIH Consensus Development Conference [21] identified *H. pylori* as the primary reason for peptic ulcer development. *H. pylori* was also evaluated by the International Agency for Research on Cancer (IARC) and was identified as a human Group 1 carcinogen (1994). *H. pylori* is associated with both gastric adenocarcinoma and gastric lymphomas. *H. pylori* is transmitted to humans through a variety of ways, including oral-oral and fecal-oral routes [9].

DEHP is a plasticizer found widely in food packaging, which often can migrate from the plastic wrapping and actually contaminate the packaged food [22]. DEHP contamination is prevalent throughout the food chain [23]. Meat, fish, dairy products, fresh fruit, and bread all exhibited 100% prevalence for DEHP contamination. Other important foods also exhibited a prevalence for DEHP contamination to a lesser extent; cereals and legumes (93%), vegetables (80%), and condiments (66%) [24]. The estimated daily intake of phthalates in the general Taiwanese population is approximately 0.1 to 309.6 $\mu\text{g}/\text{kgBW}/\text{day}$ (about 0.015 to 47.5 μM by a 60 kg adult) [15]. With PVC industry workers, the daily intake of phthalates is much higher at about 0.6–850 $\mu\text{g}/\text{kgBW}/\text{day}$ (about 0.092 to 130.6 μM by a 60 kg adult) [25]. Since the half-life of DEHP in the human body ranges between 16 and 24 hours [26], DEHP may be able to stimulate the epithelial cells of the gastrointestinal tract and accumulate in the human body via sustainably ingested of DEHP-contaminated foods. In humans, ingestion of 10 grams of DEHP (~426 μM ,

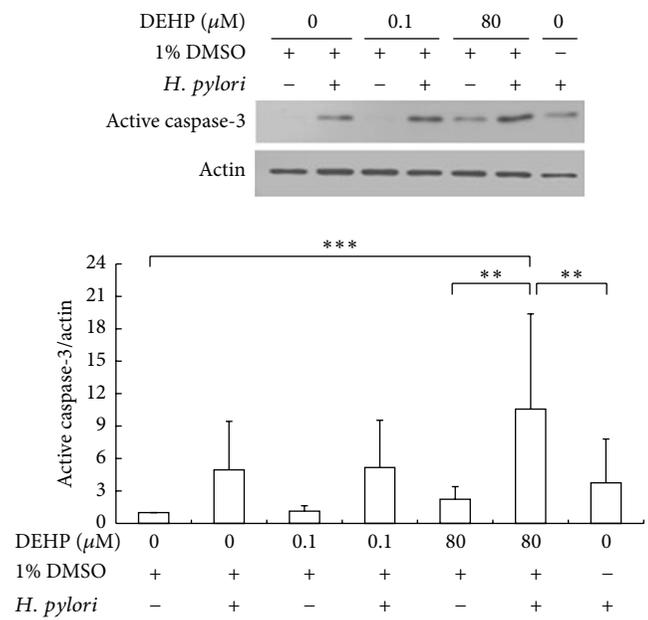


FIGURE 4: DEHP and *H. pylori* increased active form of caspase-3 protein expression after 18-hour treatment. Statistical significance was analyzed by one-way ANOVA and post hoc LSD test (** $P < 0.05$, *** $P < 0.01$, $N = 4$).

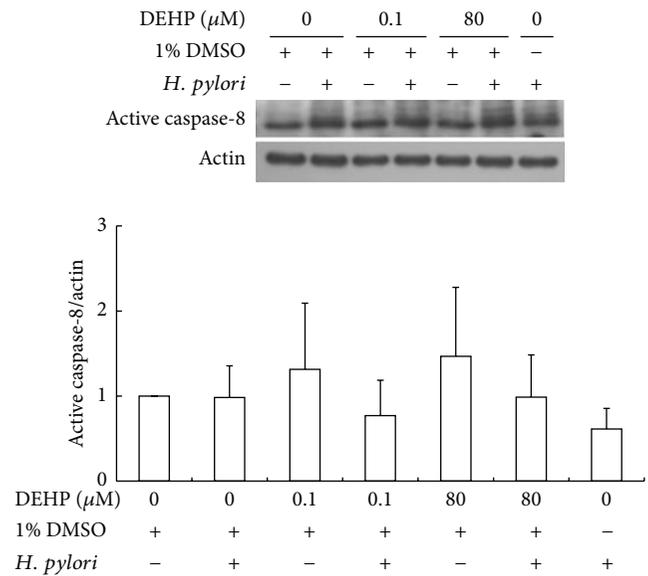


FIGURE 5: The effect of DEHP and *H. pylori* on active caspase-8 protein expression after 18-hour treatment. Statistical significance was analyzed by one-way ANOVA and post hoc LSD test ($N = 3$).

assuming 60 kg b.w.) can cause mild gastric disturbances and “moderate catharsis” [27]; however, the effects of short and long term high level exposure to DEHP are not known. In this study, the effects of *H. pylori* and DEHP were identified on gastric epithelial cells. We found that DEHP and *H. pylori* coexposure decreased AGS cell viability greater than DEHP or *H. pylori* alone (Figure 1), indicating that the combined exposure of *H. pylori* and DEHP has additive toxic effects on

TABLE 1: Summary of apoptosis-related proteins expression ratio after DEHP alone or DEHP combined with *H. pylori* (MOI = 100:1) treatment^a.

Ratio	N		Medium (1% DMSO)		Medium (1% DMSO) + Hp/AGS cells (MOI 100:1)		Medium (w/o DMSO) + Hp/AGS cells (MOI 100:1)	
			Mean	SD	Mean	SD	Mean	SD
Active caspase-3/actin	4	Vehicle	1.00	0.00	4.95	4.49		
		0.1 uM DEHP	1.14	0.50	5.17	4.36		
		80 uM DEHP	2.23	1.17	10.57	8.81		
		Only <i>H. pylori</i>					3.75	4.05
Active caspase-8/actin	3	Vehicle	1.00	0.00	0.98	0.37		
		0.1 uM DEHP	1.31	0.78	0.77	0.42		
		80 uM DEHP	1.47	0.81	0.99	0.50		
		Only <i>H. pylori</i>					0.61	0.24
Bax/Bcl-2	5	Vehicle	1.00	0.00	1.33	0.29		
		0.1 uM DEHP	1.30	0.44	1.54	0.49		
		80 uM DEHP	1.56	0.54	1.89	0.53		
		Only <i>H. pylori</i>					1.47	0.32

^aThe protein expression ratio was normalized to vehicle group.

gastrointestinal epithelial cells, potentially altering the ratio between cell proliferation and apoptosis.

The imbalance between cell proliferation and apoptosis may contribute to gastric carcinogenesis. Gastric resection specimens from patients that exhibited normal gastric mucosa contained a low number of apoptotic cells at the surface epithelium. The apoptotic number was significantly increased in cases with chronic gastritis and/or intestinal metaplasia [28]. Increased apoptosis is associated with the development of gastric carcinoma [29]. The TUNEL assay on biopsies obtained from the gastric mucosa in patient with gastric carcinoma also found multiple apoptotic cells [30]. In addition, it has been shown that the increase rate of *H. pylori*-induced apoptosis on AGS cell line was 47.0% and on a normal gastric epithelial cell line (GES-1), which is developed by Beijing Institute for Cancer Research Collection, was 113.0% [31], the flow cytometry experiment also showed the ratio of apoptosis in GES-1 cells was 10.2%~27.6% after *H. pylori* infection for 4 hours [32], and the ratio of apoptosis in AGS cells was about 18% after *H. pylori* infection for 6 hours [33]. These articles indicated that normal gastric epithelial cell might be more sensitive than AGS cell to *H. pylori* and could cause higher ratio apoptosis than AGS cell after *H. pylori* infection. Because apoptosis ratio increase is one of the possible pathways which associated with the development of gastric carcinoma [29], *H. pylori*-induced carcinogenesis might be more evident on normal gastric epithelial cell than AGS cells. In this study, we found that *H. pylori* and DEHP coexposure increased DNA fragmentation of AGS cells (Figure 2). This result implies that *H. pylori* and DEHP coexposure might induce cell apoptosis and alter the balance between cell proliferation and cell death in gastric epithelial cells, disrupting the integrity of the gastric mucosa and promoting gastric

carcinogenesis. Moreover, the ratio of *H. pylori* and DEHP coexposure was higher than *H. pylori* infection alone, further indicating that DEHP enhances *H. pylori*-induced apoptosis on AGS cells. Apoptosis has been shown that, related with the cell subpopulations of highly growth rate selection in gastric precancerous lesions and involved in the malignant transformation [34], *H. pylori* is type I carcinogen and DEHP is type 2B carcinogen in IARC classification (2000); moreover, we found DEHP and *H. pylori* exposure increased AGS cell apoptosis. Therefore, DEHP and *H. pylori*-induced AGS cell apoptosis might increase the AGS cell malignant transformation.

Previous articles also revealed that apoptosis-related proteins expression was different in various types of gastric precancerous lesions and might involve in the process of carcinogenesis and metastasis. Bcl-2 protein expression was increased in gastric premalignant lesions and decreased its expression after malignant change [34, 35]. Bax protein expression was upregulated in patient's gastric precancerous lesions after *H. pylori* infection [36]. Bax protein is also highly expressed in intestinal metaplasia regions nearby to tumors and related with induction of apoptosis [35]. Bax protein was found highly expression in gastric cancer patient's tissues and there was no difference in the tumor stage [37]. Taken together, the Bax/Bcl-2 ratio of cancer cell might increase after malignant change. Our results show that combined exposure of DEHP and *H. pylori* increased the Bax/Bcl-2 ratio of AGS cell (Figure 3), this result might imply DEHP, and *H. pylori* exposure might induce AGS cell malignant change.

Apoptosis includes two separate pathways: intrinsic and extrinsic. The intrinsic pathway is induced by the permeability loss of the mitochondrial outer membrane. The permeability loss of the mitochondrial outer membrane leads to cytochrome c release, apoptosome formation, and procaspase-9

activation. The extrinsic pathway is initiated by extracellular death ligand binding, which results in the activation of caspase-8. Both the intrinsic and extrinsic pathways activate caspase-3 and result in apoptosis [38, 39]. *H. pylori* was reported to induce gastric epithelial cell apoptosis through the activation of the extrinsic pathway [40]; however, in a different study, *H. pylori* was also reported to induce apoptosis mainly through the intrinsic pathway. Caspase-8 activation did not appear to play a major role in *H. pylori* induced apoptosis [41]. In this study, the DEHP and *H. pylori* coexposure significantly increased activated caspase-3 in AGS cells (Figure 4), but caspase-8 activation was not changed after an 18 hr exposure (Figure 5). This result is similar to previously reviewed articles, indicating that caspase-8 is not essential for DEHP or *H. pylori*-induced apoptosis and that DEHP and *H. pylori*-induced apoptosis is also likely mediated by the intrinsic pathway. Additionally, active caspase-3 was significantly higher in cells that were co-exposed to DEHP and *H. pylori* than cells exposed to DEHP or *H. pylori* alone. These results paralleled the DNA fragmentation study, indicating DEHP and *H. pylori* coexposure has an additive effect on caspase-3 activation and apoptosis of AGS cells. Moreover, caspase-3 activation plays an important role in stress-induced invasion [42], high level caspase-3 expression in the tissue sample of gastric cancer patients has been shown poor prognosis and related with gastric cancer lymph node metastasis [43]. The caspase-3 protein expression in primary gastric carcinoma was higher than metastatic gastric carcinomas [44, 45]. This study found that DEHP and *H. pylori* exposure increased the expression of active form of caspase-3 in AGS cells and induced AGS cells apoptosis, and this result implied that DEHP and *H. pylori* might enhance the ability of stress-induced invasion ability of gastric cancer. Taken together, these data not only indicate that while caspase-3 likely plays an important role in AGS cell apoptosis induced by the combined DEHP and *H. pylori* exposure, but also imply the additive effect on malignant transformation of AGS cell after DEHP and *H. pylori* exposure.

In conclusion, this paper reports that DEHP and *H. pylori* coexposure can regulate Bax and Bcl-2 protein expression to increase Bax/Bcl-2 ratio, activate caspase-3 protein, and enhance AGS cellular apoptosis. These results provide new information about the carcinogenetic effect of DEHP and *H. pylori* coexposure on gastric epithelial cells, which may, with further research, suggest a possible mechanism, in which DEHP enhances carcinogenesis when combined with a known carcinogen. Therefore, a further investigation is necessary to understand the underlying mechanism by which DEHP and *H. pylori* can induce carcinogenesis and metastasis in gastric epithelial cells.

Conflict of Interests

The authors declare that there is no conflict of interests.

Acknowledgments

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Clinical Study

Eradication of *Helicobacter pylori* Is Associated with the Progression of Dementia: A Population-Based Study

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Objective. To evaluate the effect of eradication of *Helicobacter pylori* (*H. pylori*) on the progression of dementia in Alzheimer's disease (AD) patients with peptic ulcer. **Methods.** Participants with the diagnosis of AD and peptic ulcer were recruited between 2001 and 2008. We examined the association between eradication of *H. pylori* and the progression of AD using the multiple regression models. Medication shift from Donepezil, Rivastigmine, and Galantamine to Memantine is defined as progression of dementia according to the insurance of National Health Insurance (NHI) under expert review. **Results.** Among the 30142 AD patients with peptic ulcers, the ratio of medication shift in AD patients with peptic ulcers is 79.95%. There were significant lower incidence comorbidities (diabetes mellitus, hypertension, cerebrovascular disease, coronary artery disease, congestive heart failure and hyperlipidemia) in patients with *H. pylori* eradication as compared with no *H. pylori* eradication. Eradication of *H. pylori* was associated with a decreased risk of AD progression (odds ratio [OR] 0.35 [0.23–0.52]) as compared with no *H. pylori* eradication, which was not modified by comorbidities. **Conclusions.** Eradication of *H. pylori* was associated with a decreased progression of dementia as compared to no eradication of *H. pylori* in AD patients with peptic ulcers.

1. Introduction

Alzheimer's disease (AD) is a common neurodegenerative disorder for which causes are diverse, and it involves similar neuroinflammation cascade as prion disease [1]. Cerebral amyloid deposits are colocalized with a broad variety of inflammation-related proteins (complement factors, acute-phase protein, and proinflammatory cytokines) and clusters of activated microglia [2]. Currently, identified risk factors

of AD include age, sex, plasma homocysteine level, and genetic factors like apolipoprotein E allele $\epsilon 4$ [3, 4]. Several studies have shown the association between infection and AD, including HSV-1, *Chlamydia pneumoniae*, spirochetes, and *Helicobacter pylori* (*H. pylori*) [5–8]. As for *H. pylori* infection, previous case-control studies found an association between *H. pylori* and AD. An impressive intervention study has shown positive results that the *H. pylori* eradication may improve the cognitive function outcome within two years,

but the sample size of case (28 patients) and controls (16 patients) might be small for application to general population [9]. Additionally, some agents like statins, inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, have also shown to potentially attenuate neuroinflammatory processes and play a role in halting the degeneration process of AD [10–13]. A recent case-control study also showed preliminary results that AD patients with *H. pylori* infection may be more cognitively impaired. Roubaud-Baudron et al. found higher CSF cytokine (TNF- α , IL-8) and significantly positive correlation between *H. pylori* immunoglobulin level and homocysteine level and they concluded that the impact of *H. pylori* infection on AD course may be attributed to cerebrovascular lesions and neuroinflammation [14]. These observations led to the hypothesis that eradication of *H. pylori* infection modulating neuroinflammatory process may have a protective role for AD. Given that Taiwan's National Health Insurance Reimbursement Policy requested physicians to perform eradication of *H. pylori* infection depending on gastrointestinal endoscopy biopsy with or without ¹³C-urea breath test and neurologists to treat worsening of AD patients with anti-cholinesterase treatment according to repeated neuropsychological assessment and detailed expert review, we were allowed to observe the impact of eradication of *H. pylori* on the AD course.

2. Methods

2.1. Data Source. The database used in this study included one million randomly selected subjects from the 1996–2007 Taiwan National Health Insurance Research Database (NHIRD), which was developed for research purposes. The NHIRD is a research database developed at the National Health Research Institute, with linked data from the demographic and enrollment records, hospital claims, ambulatory care visits, and pharmacy dispensing claims from hospitals, outpatient clinics, and community pharmacies. Our source population comprised all beneficiaries from the Longitudinal Health Insurance Database 2005 who were at least 50 years of age on January 1, 2001. There were no statistically significant differences in age, gender, or average insured payroll-related amount between the sample group and all enrollees.

Study Population. From the NHID, AD patients who were collected from outpatient pharmacy database between January 1, 1997, and December 31, 2004, with a primary diagnosis of dementia (Classifications of Diseases-9 codes: 290.xx) and regularly taking anticholinesterase medications (include donepezil, rivastigmine, or galantamine according to anatomical therapeutic chemical (ATC) classification system codes provided in Supplemental Table 1 in the supplementary material available online at <http://dx.doi.org/10.1155/2013/175729>) for more than 3 months. We then selected AD patients with the diagnosis of peptic ulcer (Classifications of Diseases-9 codes: 531–534, A-code: A534). Patients who received *H. pylori* eradication therapy and those who did not receive *H. pylori* eradication therapy were classified into two subgroups. Due to Taiwan's National Health Insurance Reimbursement Policy request,

worsening of neuropsychological assessment including minimal status exam may shift anticholinesterase medications (donepezil, rivastigmine, or galantamine) to memantine and defined as worsening of dementia; AD patients who shift or did not shift anti-cholinesterase medications were analyzed separately. Comorbidities were defined as diseases diagnosed before the index outpatient clinic visit.

2.2. *H. pylori* Eradication Method. *H. pylori* eradication with triple or quadruple therapy was defined as proton pump inhibitor or H₂ receptor blocker, plus clarithromycin or metronidazole, plus amoxicillin or tetracycline, with or without Bismuth (details for all eligible *H. pylori* eradication regimens are reported previously) [15]. These drug combinations were prescribed within the same prescription order, and the duration of therapy was between 7 and 14 days. One year was chosen as the cutoff value based on the distribution of *H. pylori* eradication date after index hospitalization.

2.3. Covariate Ascertainment and Adjustment. We used inpatient and outpatient diagnosis files and prescription files during the 12-month period before the index date to ascertain patients' history of hypertension diabetes mellitus, cerebrovascular disease, coronary artery disease, congestive heart failure, and hyperlipidemia (ICD-9-CM codes provided in Supplemental Table 2); we also collected patient information on age, sex, and resource utilization (number of outpatient visits, number of hospitalizations, number of laboratory test measurements) 12 months prior to the index date.

2.4. Statistical Analysis. Baseline characteristics, comorbidities, and medication use were presented. For all cohort members, we computed their person days of followup in each anti-cholinesterase medication category. We examined the effect of *H. pylori* eradication therapy on risk of shifting anticholinesterase medications by comparing the occurrence of shifting medications after the *H. pylori* eradication therapy among AD patients with peptic ulcers. Multiple regression model was used to calculate the odds ratios (ORs) and their 95% CIs.

3. Results

After excluding subjects who did not meet our study criteria, a total of 30142 AD patients were included in the analysis (Figure 1). A total of 1538 AD patients with peptic ulcer were then selected and classified into two groups: with *H. pylori* eradication ($n = 675$) and without *H. pylori* eradication ($n = 863$). Among these two groups enrolled in our study, several baseline characteristics, including diabetes mellitus, hypertension, cerebrovascular disease, congestive heart failure, coronary artery disease, and hyperlipidemia, were higher in frequency in patients without *H. pylori* eradication (Table 1). According to our previous study, the eradication rate of *H. pylori* was 89.4% to 90.5% [16]. Thus, it was estimated that 90% of our patients ($n = 675$) had successful eradication of *H. pylori*. Our results would underestimate the association

TABLE 1: Basic demographic data of demented patients with peptic ulcer.

	1538 demented patients with PU (ICD-9:290.xx & 531-534)		P value
<i>H. pylori</i> eradication	Yes, <i>n</i> = 675	No, <i>n</i> = 863	
Age, yr	79.41 ± 9.64	76.07 ± 11.59	
Female, %	338 (50.00)	475 (55.77)	0.0528
Diabetes mellitus, %	46 (6.81)	161 (18.65)	<0.0001
Hypertension, %	85 (12.59)	308 (35.56)	<0.0001
Cerebrovascular disease and TIA, %	57 (8.44)	277 (32.09)	<0.0001
Congestive heart failure, %	32 (4.74)	102 (11.81)	<0.0001
Coronary artery disease, %	42 (6.22)	185 (21.43)	<0.0001
Hyperlipidemia, %	45 (6.66)	159 (18.42)	<0.0001

PU: peptic ulcer. TIA: transient ischemic attack.

P value, by student's *t*-test.

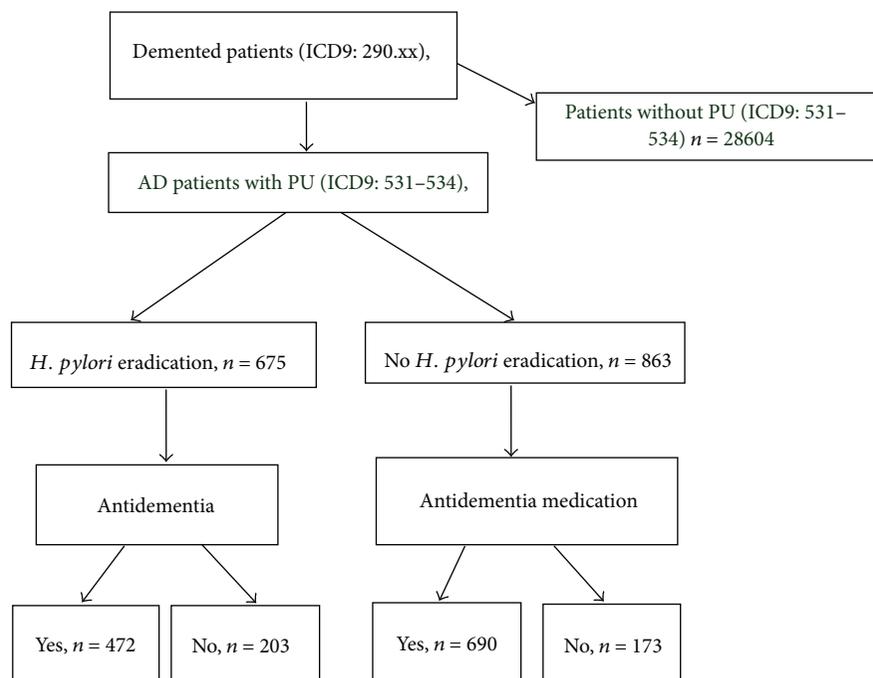


FIGURE 1: Flow chart of the study cohort assembly from prescriptions in Taiwan's National Health Insurance Research Database. Taiwan National Health Insurance Database, 1 million subtract from 23 million.

between eradication therapy of *H. pylori* and the progression of AD.

As compared with no *H. pylori* eradication, *H. pylori* eradication was associated with a decreased risk of changing anticholinesterase medication with the OR of 0.58 (95% CI 0.46–0.74). The protective association of *H. pylori* eradication persisted with OR of 0.34 (95% CI 0.23–0.52) after adjustment of comorbidities (Table 2).

4. Discussion

Our study demonstrated that eradication therapy of *H. pylori* has a decreased association with AD progression as compared to no *H. pylori* eradication in AD patients with peptic ulcer, after adjusting for age, sex, comorbidities, and other

potential confounder medications. We also observed a higher frequency of comorbidities in patients without eradication therapy of *H. pylori*. Previous reports also suggested the association between *H. pylori* infection and diabetes mellitus, hypertension, and cerebrovascular disease [17, 18]. In patients with diabetes mellitus, *H. pylori* infection or seropositivity not only increases the risk of atherosclerosis and cardiovascular disease but also contributed to promoting insulin resistance and increased microalbuminemia [18, 19].

Chronic *H. pylori* infection has shown to increase gastric pH level of gastric juice and thus leads to reduced folate absorption and increased blood homocysteine level, both of which would result in the damage of vascular endothelial cells and increased the risk of atherosclerosis [20–23]. However, the link between cerebrovascular disease and *H. pylori*

TABLE 2: The effect of *H. pylori* eradication on the progression of dementia.

	1538 demented people with PU (ICD-9:290.xx & ICD-9:531-534)		Percentage, %	OR, (95% CI)	Adjusted OR, (95% CI)
	<i>H. pylori</i> eradication Yes	No			
Medication change (+)	472	690	79.95	0.58 (0.46–0.74)	0.34 (0.23–0.52)
Medication change (–)	203	173	20.05	1	1

PU: peptic ulcer. OR: odds ratio. We estimated odds ratio of progression of dementia by χ^2 test.

We estimated adjusted odds ratio by multiple linear regression, adjusted with age, gender, diabetes mellitus, hypertension, cerebrovascular disease and TIA, congestive heart failure, coronary artery disease, and hyperlipidemia.

infection still needs prospective studies [14, 24]. Our study may provide some support for the hypothesis of *H. pylori* infection causing atherosclerosis and risk of cerebrovascular disease.

Indeed, vascular risk factors, including adult-onset diabetes mellitus, hypertension, atherosclerosis disease and atrial fibrillation, are known to increase the risk of AD [25]. Cerebral vascular dysfunction may cause the accumulation of amyloid-beta (Abeta) protein and neuroinflammation in animal model, and reduction of fibrillar Abeta protein deposition may ameliorate the neuroinflammation [26, 27]. Neuroinflammation disrupting the blood brain barrier, together with fibrinogen, may be one of the contributing factors for familial cerebral amyloid angiopathy and Alzheimer's disease [28, 29]. It is plausible to propose that antibacterial treatment of chronic inflammation caused by *H. pylori* or other pathogens like spirochete may reduce neuroinflammation and thus prevent dementia [7].

The strength of our study is the enrollment of a nationally representative cohort of a large sample size. The information regarding anticholinesterase medications and eradication therapy of *H. pylori* is obtained by linking to the NHI pharmacy database under the Reimbursement Policy request of NHI to reduce the possibility of duplication or misclassification. Furthermore, covariates including underlying diseases (especially diabetes mellitus), were taken into consideration. However, there are several limitations. First, although we analyzed health care records from a national representative dataset of 1 million people, there were still few AD cases to allow us to have a precise estimation. Second, we did not adjust the use of medications that potentially may affect AD risk such as statins, NSAID, antidiabetic agents, calcium channel blockers, and neuroleptic agents. Third, we were not able to assess the genotype of apolipoprotein E allele $\epsilon 4$, which cement the solid relevance in late-onset AD [30–32]. Fourth, our diagnosis of AD was based on the diagnosis code from the NHI database; therefore, we were not able to distinguish between AD and mixed type dementia. Nonetheless, a stringent policy from NHI validates our diagnosis by expert review before the use of anticholinesterase medications. However, given that all the medical information from the NHI database was deidentified due to ethical privacy concern, we could not recognize all the AD-diagnosed subjects in our study and therefore did not have the opportunity to review all their medical charts. Last, we could not exclude the possibility that the observed association was due to sick-stopper effect (nonadherence to

medication due to higher risk) or protopathic bias (less AD symptoms may increase the awareness of the importance of eradication therapy). Further longitudinal study including measures of neuropsychiatric assessment over time is needed to clarify the interrelated roles of cognition, eradication therapy of *H. pylori*, and AD.

5. Conclusion

We observed that eradication therapy of *H. pylori* had a decreased association with AD progression compared with no eradication therapy among AD patients with peptic ulcer. Further long-term follow-up study is needed to confirm the potential beneficial role of antibacterial therapy of *H. pylori* in AD.

Acknowledgments

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Research Article

Helicobacter pylori Infection and Anemia in Taiwanese Adults

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Background. Chronic *Helicobacter pylori* infection and iron-deficiency anemia (IDA) are common in adults. Although the most common causes of IDA usually arise from the gastrointestinal tract, the association between chronic *Helicobacter pylori* infection and anemia remains unclear. **Aim.** To evaluate the association of chronic *Helicobacter pylori* infection and IDA. **Materials and Methods.** We enrolled 882 patients from January 2010 to April 2013. The status of *Helicobacter pylori* (H.p) infection was confirmed and blood samples from the same participants were taken on the same day to check the level of hemoglobin, serum iron, ferritin, and total iron-binding capacity (TIBC). **Results.** No significant difference was noted from the demographic data. The average level of hemoglobin (Hb) was not different between negative and positive groups, pos 13.57 g/dL versus neg 13.65 g/dL ($P = 0.699$). Although the levels of serum IDA related parameters were expected in positive group (lower serum iron and ferritin and higher TIBC) these differences did not reach statistical significance ($P = 0.824$ for iron, $P = 0.360$ for ferritin, and $P = 0.252$ for TIBC). **Conclusion.** Chronic *Helicobacter pylori* infection is not attributed to IDA. The levels of hemoglobin, serum iron and ferritin, and TIBC remain unaffected after chronic H.p infection. Large-scale clinical studies are needed to prove the association.

1. Introduction

Chronic *Helicobacter pylori* (H.p) infection is responsible for many alimentary tract disorders, including gastroduodenal

ulcer, atrophic gastritis, intestinal metaplasia, gastric mucosa-associated lymphoid tissue lymphoma (MALT lymphoma), and gastric adenocarcinoma [1]. Furthermore, it has been implicated in some extragastric diseases, such as unexplained

iron-deficiency anemia (IDA), idiopathic thrombocytopenic purpura (ITP), and vitamin B12 deficiency [2, 3].

Iron-deficiency anemia (IDA) is the most common cause of anemia in the world and 500 to 600 million people are affected. IDA is also the most common nutritional deficiency in undeveloped and developed worlds and possibly the most common organic disorder in clinical practice [4, 5]. Anemia is a common manifestation of various etiologies, for example, iron-deficiency, vitamin B12 deficiency, folic acid deficiency, chronic illness, gastrointestinal bleeding, and so forth. Generally, it is attributed to three different pathogenic processes: (1) marrow production defects (hypoproliferation); (2) red cell maturation defects (ineffective erythropoiesis); and (3) decreased red cell survival (blood loss/hemolysis). Consequently, chronic *H.p* infection is likely to result in anemia. For example, chronic inflammation due to chronic *H.p* infection is one of the causes which have been related marrow production defects (hypoproliferation) [6]. Iron-deficiency anemia (IDA) is a common cause which occurs in 2–5% of adult males and postmenopausal females in developed countries [7, 8], and most of them resulted from gastrointestinal lesions. Chronic *H.p* infection usually causes a wide range of gastrointestinal mucosal lesions, such as chronic erosive gastritis, and is very likely to contribute to IDA in affected patients. In addition, chronic *H.p* infection frequently results in atrophic gastritis, which leads to hypochlorhydria, which is the underlying cause for decreased iron absorption and increased iron uptake and utilization by the bacteria [4].

Despite all the assumptions, no strong evidence from clinical studies is available. Therefore, we hypothesize that chronic *H.p* infection is related to anemia and have conducted this prospective study to clarify the association between *H.p* infection and anemia.

2. Patients and Methods

2.1. Study Design and Patients. Initially, one thousand two hundred and eighteen patients (489 men and 729 women) were enrolled from gastroenterology clinics of three different hospitals, including Kaohsiung Medical University Hospital, Kaohsiung Municipal Hsiao-Kang Hospital, and Kaohsiung Municipal Tatung Hospital, from January 2010 to April 2013. All patients received the esophagogastroduodenoscopy (EGD) examination, and endoscopic biopsy from gastric mucosa was undertaken for confirmation of *H.p* infection. Exclusion criteria for *H.p* infection included use of antibiotics, bismuth, or proton pump inhibitor (PPI) within 4 weeks, previous gastric surgery, and history of eradication of *H.p*. Besides blood was drawn from all of them for hemoglobin, serum iron, serum total iron-binding capacity (TIBC) and serum ferritin checks on the same day. Exclusion criteria for anemia included past history of anemia with known etiology other than *H.p*, known hematologic disorder causing anemia, evident gastrointestinal bleeding within one month, and evident blood loss within one month. After exclusion, we enrolled 882 cases for further analysis. As for the evaluation of chronic *H.p* infection and iron-deficiency

anemia, we just enrolled 770 cases due to the patients' unwillingness to be checked for serum iron level.

2.2. Diagnosis of *H. pylori* Infection. We used culture, histology, rapid urease test, and ¹³C-urea breath test (UBT) in this study. The Columbia blood agar plate is made use of for culture for endoscopic biopsy specimens. The culture demonstrated positive if one or more colonies showed Gram-negative, oxidase (+), catalase (+), urease (+), or spiral or curved rods in morphology. We also evaluated the presence of *H. pylori* in the histology of gastric biopsy specimens by experienced pathologists. If the color of rapid urease test (sensitivity 93–97%, specificity 98%) [9], CLO test (Delta West Bentley, WA, Australia), turned pink or red at room temperature 6 hours after the EGD examination, it was interpreted as positive. The ¹³C-urea breath test used in this study was from the Institute of Nuclear Energy Research, Taiwan. The definition of positive *H. pylori* infection was that either culture was positive or at least two positive results of rapid urease test, histology, or UBT [10, 11].

2.3. Definition of Anemia. Anemia was defined as serum hemoglobin (Hb) <14 g/dL in males and <12 g/dL in females. The definition of iron-deficiency anemia (IDA) was serum iron <30 µg/dL and total iron-binding capacity (TIBC) >400 µg/dL [6]. We also analyzed the association between chronic *H.p* infection and iron-deficient erythropoiesis as definition of serum iron <50 µg/dL and total iron-binding capacity (TIBC) >380 µg/dL [6]. Normal ranges of serum iron, TIBC, and ferritin are 45–182 µg/dL (male)/28–170 µg/dL (female), 257–421 µg/dL (male)/254–450 µg/dL (female), and 24–336 ng/mL (male)/11–307 ng/mL (female), respectively.

2.4. Statistical Analysis. The demographic characteristics and average serum iron, TIBC, and ferritin levels were analyzed by Student's *t*-test. The relationships between *H.p* infection and anemia and IDA and iron-deficient erythropoiesis were analyzed by Chi-square test. Statistical significance was considered as $P < 0.05$.

3. Results

3.1. Demographic Characteristics. A total of 882 patients were enrolled into the study. The average ages of negative and positive *H.p* infection groups were 57.6 ± 12.7 and 57.5 ± 12.4 years old, respectively, ranging from 21 to 88 years old (Table 1). No significant difference of the demographic characteristics, including age, sex, cigarette smoking, hypertension and cerebrovascular disease, was demonstrated between negative and positive *H.p* infection groups.

3.2. *H.p* Infection and Anemia. The average level of serum hemoglobin (Hb) was 13.65 g/dL in negative group and 13.57 g/dL in positive group. We analyzed the relationship between *H.p* infection and mean Hb and anemia (defined as serum Hb < 14 g/dL in males and <12 g/dL in females), and

TABLE 1: Demographic characteristics between negative and positive H.p infection groups.

	H.p (-) (n = 762)	H.p (+) (n = 120)	P value
Age(years)			
Mean ± SD	57.6 ± 12.7	57.5 ± 12.4	0.936
Sex			0.939
Male	302 (39.6%)	48 (40%)	
Female	460 (60.4%)	72 (60%)	
Smoking	63 (8.3%)	12 (10%)	0.528
Hypertension	184 (24.1%)	30 (25%)	0.84
Cerebrovascular disease	65 (8.5%)	8 (6.7%)	0.492

*H.p (-) as negative H.p infection and H.p (+) as positive H.p infection.

TABLE 2: The relationship between H.p infection and hemoglobin (Hb) and anemia.

	H.p (-) (n = 762)	H.p (+) (n = 120)	P value
Mean Hb g/dL	13.65	13.57	0.699
Anemia (-)	599 (78.6%)	99 (82.5%)	0.397
Anemia (+)	163 (21.4%)	21 (17.5%)	

Anemia: male: Hb < 14 g/dL, female: Hb < 12.

no significant difference was noted, even though the level of Hb was lower in the positive group ($P = 0.699$) (Table 2).

3.3. H.p Infection and Iron, Total Iron-Binding Capacity (TIBC), Ferritin, Iron-Deficiency Anemia (IDA) and Iron-Deficient Erythropoiesis. We also evaluated the serum iron, total iron-binding capacity (TIBC), and ferritin of the patients after the enrollment. In the positive H.p infection group, several lines of evidence of IDA, such as lower serum iron, lower serum ferritin, and higher TIBC, were demonstrated but did not reach statistical significance (Table 3). Furthermore, we performed subgroup analyses as serum iron <30 $\mu\text{g/dL}$, TIBC >400 $\mu\text{g/dL}$, and ferritin <15 ng/mL, according to the standard definition of IDA. Although more patients in the positive groups had higher TIBC and lower ferritin, there was no significant difference after subgroup analysis (Table 3).

In this study, we enrolled four cases of IDA in the negative H.p infection group but no case in the positive group. With respect to iron-deficient erythropoiesis, twenty and five cases were collected into the negative and positive H.p infection groups individually. Again, no statistical significance was noted between the negative and positive groups (Table 4).

4. Discussion

In this study, among the 882 patients, we showed no significant association between chronic *Helicobacter pylori* (H.p) infection and anemia. Although we observed lower levels of hemoglobin in the positive H.p infection group, there was no

TABLE 3: The association between H.p infection and iron, ferritin, and TIBC.

	H.p (-) (n = 660)	H.p (+) (n = 110)	P value
Mean iron ($\mu\text{g/dL}$)	95.28	94.41	0.824
Mean ferritin (ng/mL)	138.07	127.41	0.360
Mean IBC ($\mu\text{g/dL}$)	330.98	337.68	0.252
Iron < 30	15 (2.3%)	2 (1.8%)	0.765
Iron 30	645 (97.7%)	108 (98.2%)	
TIBC > 400	63 (9.5%)	14 (12.7%)	0.301
TIBC < 400	597 (90.5%)	96 (87.3%)	
Ferritin < 15	39 (5.9%)	7 (6.4%)	0.849
Ferritin > 15	621 (94.1%)	103 (93.6%)	

TABLE 4: The association between H.p infection and IDA and iron-deficient erythropoiesis.

	H.p (-) (n = 660)	H.p (+) (n = 110)	P value
IDA (+) (iron < 30, TIBC > 400)	4	0	
IDA (-)	656	110	
Iron-deficient erythropoiesis (+) (iron < 50, TIBC > 380)	20	5	0.385
Iron-deficient erythropoiesis (-)	640	105	

statistical significance. Also, we showed no significant association between chronic H.p infection and iron-deficiency anemia (IDA), despite lower levels serum iron and ferritin and higher levels of TIBC levels in the positive H.p infected group. There was no significant association between chronic H.p infection and iron-deficient erythropoiesis, either.

Presumably, chronic H.p infection is very likely to cause anemia, given its nature of chronic infection in adults and predisposition to gastrointestinal mucosal lesions, both of which have been attributed as the common causes of anemia [12]. Our data have agreed with previous reports on no significant association between chronic H.p infection and anemia [13–16]. Some studies have reported decreased iron store in positive H.p infection populations [17–20]. We also noted all the features of iron storage, such as lower levels of serum iron and ferritin and higher levels of total iron-binding capacity (TIBC). However, we failed to show the statistical significance.

Serum ferritin is the major storage protein for iron and the most powerful parameter for diagnosis of iron-deficiency anemia without inflammation [7]. Concomitant inflammation can greatly affect the level of serum ferritin. The level of ferritin for iron-deficiency varies from 12–15 ng/mL without concomitant inflammation to more than 50 ng/mL with concomitant inflammation [21, 22]. The levels of serum ferritin are liable to change under many conditions, including chronic inflammation, hyperthyroidism,

malignancy (leukemia, Hodgkin's disease), and even type 2 diabetes mellitus. Although many studies reported a lower level of serum ferritin in patients with chronic H.p infection, our data failed to show the significant association between the lower level of serum ferritin and chronic H.p infection in our study.

In this study, there are several limits. The relatively small sample size is not able to portray the relatively subtle difference among all the parameters. We did not exclude most of the concomitant conditions which would confound the parameters, such as the level of ferritin. Therefore, more detailed studies are needed and may help to delineate the changes in levels of hemoglobin, iron, and ferritin after chronic H.p infection and the possible reversal after H.p eradication.

Conflict of Interests

All authors have no conflict of interests to declare.

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Research Article

Helicobacter pylori Infection in Dialysis Patients: A Meta-Analysis

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Background. Infection with *Helicobacter pylori* contributes to the etiopathogenesis of various extragastrointestinal conditions, yet its etiological association with either symptomatic or asymptomatic dialysis patients remains inconclusive. **Methods.** Two researchers working independently conducted a literature search of the online databases PubMed, EMBase, ScienceDirect, and Cochrane Central Register of Controlled Trials to identify relevant articles to the end of 2012. Case-control and cross-sectional studies that met the inclusion criteria were included. **Results.** Fifteen studies involving 1237 dialysis patients and 1568 controls with normal renal function were included. Compared with normal controls, dialysis patients overall were associated with a relatively lower risk of *H. pylori* infection though not statistically significant. A significant inverse association was found between *H. pylori* prevalence and duration of treatments in those who were dialyzed >4 years (odds ratio 0.28; 95% confidence interval 0.22–0.36, $P < 0.00001$). No relationship between *H. pylori* status and duration of dialysis was observed in CRF patients. There were no significant differences in endoscopic features between patients and controls. **Conclusions.** Our meta-analysis found no evidence of a significant association between infection with *H. pylori* and dialysis overall, whereas long-term treatments of more than four years had a significant protective effect.

1. Introduction

Helicobacter pylori, an infectious organism, is present in about 50% of the global population, and the infection levels exceed 70% in some developing areas [1]. Infection with *H. pylori* has been implicated not only in the etiopathogenesis of gastrointestinal disease, such as gastritis, ulcerative diseases, low-grade mucosa-associated lymphoid tissue lymphoma, and gastric malignancies [2], but also in various extragastrointestinal conditions, among them chronic renal disease [3].

From 25% to 75% of chronic renal failure (CRF) patients who receive hemodialysis or continuous ambulatory peritoneal dialysis (CAPD) for long periods experience gastrointestinal troubles [4]. It has been postulated that high urea concentration makes the gastric mucosa of these patients more susceptible to colonization by *H. pylori* [5]. However, an etiological association between *H. pylori* and either symptomatic or asymptomatic dialysis patients remains inconclusive.

The prevalence of *H. pylori* infection in CRF patients may be as high as 64% and significantly higher in dialysis

patients than in normal controls [6–9]. Others [10–12] report quite the opposite. Many factors would seem to contribute to the inhibition of *H. pylori* growth in the stomach of CRF patients (e.g., higher levels of proinflammatory cytokines, impaired immune system, increased pH, higher blood urea levels, and antibiotic treatment). Nevertheless, some studies [13–19] found no difference in the prevalence of *H. pylori* infection between patients on dialysis and healthy controls, leading to the conclusion that the level of urea is not a risk factor predisposing to *H. pylori* infection in this population. Because of these conflicting reports, the seriousness of *H. pylori* infection in dialysis patients remains unclear.

The number of dialysis patients increases by 7% annually [20], and it is therefore imperative to resolve some important issues concerning *H. pylori* infection in dialysis patients. The present study is a meta-analysis, designed to help clarify the prevalence of *H. pylori* in CRF patients as well as the relationship between dialysis duration and the prevalence of *H. pylori*. In addition, *H. pylori* status in CRF patients and the course of dialysis will be discussed.

2. Materials and Methods

2.1. Literature Sources and Searches. We systematically searched the databases MEDLINE, EMBASE, ScienceDirect, and Cochrane Central Register of Controlled Trials (CENTRAL) for relevant articles and abstracts published in English (ending 31 December 2012). Terms and keywords used to identify articles in Medical Subject Headings (MeSH) were *Helicobacter pylori*/*H. pylori*, and dialysis (“*Helicobacter pylori*” OR “*H. pylori*”) AND “dialysis”). Two reviewers (MG and SPX) manually screened each eligible article’s title, abstract, and full text to independently determine if the article met the inclusion criteria (below). Differences between the reviewers were solved by consensus.

2.2. Inclusion and Exclusion Criteria. For inclusion in the meta-analysis, case-control or cross-sectional studies had to report data on the rate of *H. pylori* infection in patients with and without dialysis and include a control group with normal renal function; base diagnosis of *H. pylori* infection on histology (e.g., Giemsa stain or Warthin-Starry method), culture, immunoglobulin G (IgG) antibody detection, rapid urease test, or urea breath test; concern human subjects only; and be published in English. Studies were excluded that were case reports, observational studies without control groups, review of the literature, or duplicated reports; if data on *H. pylori* infection in the dialysis group or control group was incomplete or unavailable; or if subjects had a history of drug use for antibiotics, H₂ blockers, proton pump inhibitors, or bismuth within 4 weeks.

2.3. Data Extraction. Two independent reviewers extracted the information from the included articles. Discrepancies in the extraction were resolved by mutual discussion. For each study, the following data were collected: author; publication year; country; study design; basic characteristics of patients including number of patients with and without dialysis and type and duration of dialysis; detection methods for *H. pylori* infection and endoscopic abnormalities.

2.4. Data Analysis. The software Review Manager (RevMan, version 5.1, Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2011) was used to analyze the data. We arranged eligible articles chronologically, starting with the earliest. The odds ratios (ORs) and their 95% confidence intervals (CIs) for major outcomes were estimated in a fixed model or random model for each study. Statistical heterogeneity was evaluated with the I^2 statistic, and $I^2 > 50\%$ indicated substantial heterogeneity [29], in which case the condition random effects model was used. The differences were considered statistically significant when a P value was less than 0.05.

3. Results

3.1. Basic Information and Characteristics. The literature search initially yielded 152 articles relevant to the topic (Figure 1). Eighty-eight of these were excluded for not meeting the inclusion criteria. The full texts of the remaining 64

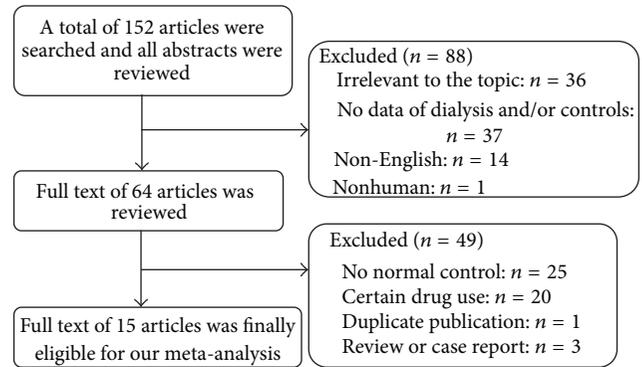


FIGURE 1: Flow chart of the eligibility selection process.

citations were carefully reviewed. Ultimately, 49 of the 64 were excluded due to the use of certain drugs within 4 weeks or for meeting any other of the exclusion criteria. This process left 15 qualified essays (Table 1).

3.2. Overall Analysis. These 15 articles comprised 1237 dialysis patients and 1568 controls with normal renal function. Since I^2 was greater than 50%, a random model was applied. Pooled data showed that there was no difference in *H. pylori* prevalence between the dialysis (hemodialysis and CAPD) patients and normal controls (OR = 0.86, 95% CI: 0.52–1.42, $P = 0.55$; Figure 2(a)). A subanalysis showed no difference in *H. pylori* infection between patients receiving hemodialysis and the control group (OR = 1.11, 95% CI: 0.69–1.81, $P = 0.66$; Figure 2(b)). A funnel plot indicated that there was no publication bias (Figure 3).

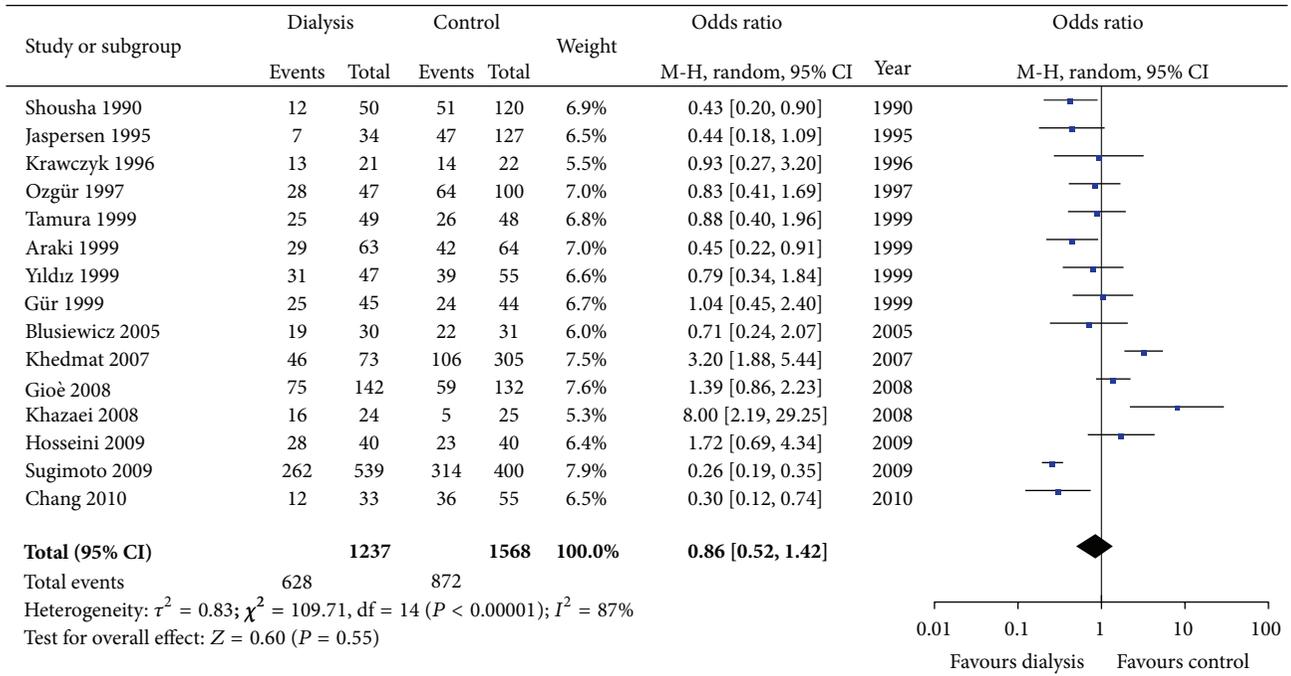
In this meta-analysis, various methods were adopted to confirm *H. pylori* infection as stated previously. As we all know, IgG antibody detection cannot judge present infection of *H. pylori*, since serum antibodies specific to *H. pylori* would still remain for several months after successful eradication, nevertheless; serology is the only test which is not affected by local changes in the stomach that could lead to a low bacterial load and to false negative results of the other tests and it is the third method commonly used as a noninvasive method to diagnose *H. pylori* infection [30]. In order to exclude the probability that different methods for *H. pylori* detection would lead to different outcomes, we chose to exclude 2 articles [11, 22] which only utilized IgG to detect *H. pylori* infection. However, subsequent analysis again found no differences between the two groups (Figure 4). Still, we wanted to detect if other detection methods like rapid urease test (RUT) would influence the overall analysis, while more than one kind of detection method was involved in the other studies included in our meta-analysis. The data of other detection methods cannot be analyzed separately.

3.3. Effect of Dialysis Duration on *H. pylori* Prevalence. Some studies have indicated that the rate of *H. pylori* infection decreases over a prolonged course of hemodialysis. Hence we performed a subgroup meta-analysis of *H. pylori* infection and the duration of dialysis (Figures 5(a) and 5(b)). Those who underwent dialysis longer than four years [4, 11, 28]

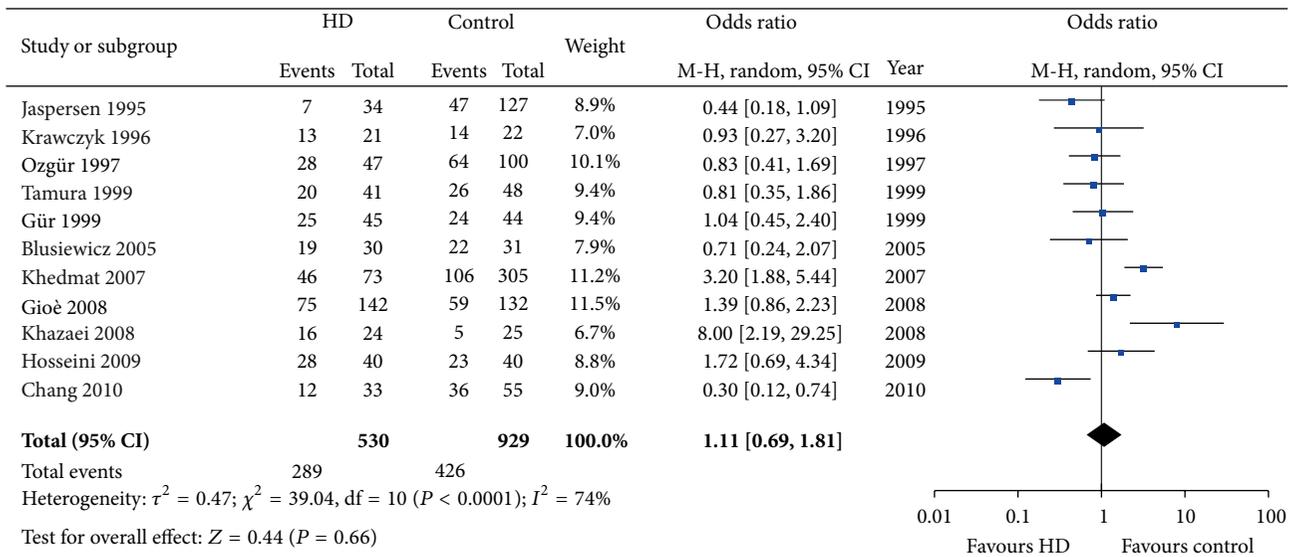
TABLE 1: Basic information of eligible articles.

Author (ref.)	Year	Country	Study design	Age, y	Test confirming infection	Duration of dialysis, m	Dialysis type, n	HP(+), n
							HD	Controls
								Dialysis
Shousha et al. [13]	1990	UK	Case-control	HP(+) 54 ± 14.3 HP(-) 48 ± 14.2	Warthin-Starry, Giemsa	NG	NG	51/120
Jaspersen et al. [10]	1995	Germany	Case-control	58.2 ± 12.6	Urease test, Giemsa	NG	7/34	47/127
Krawczyk et al. [14]	1996	Poland	Case-control	36.8 ± 13.2	Urease test, Giemsa	28 ± 12.2	13/21	14/22
Ozgür et al. [15]	1997	Turkey	Case-control	37.27 ± 14.08	Urease test	28.87 ± 28.92	28/47	64/100
Gür et al. [21]	1999	Turkey	Case-control	HP(+) 35.1 ± 4.2 HP(-) 32.5 ± 5.3	Urease test, histology	HP(+) 21.2 ± 16.4 HP(-) 21.8 ± 11.4	25/45	24/44
Araki et al. [4]	1999	Japan	Case-control	57.4 ± 12.8	Histology, culture	91.2 ± 62.4	NG/54	42/64
Yıldız et al. [22]	1999	Turkey	Cross-sectional	36.6 ± 15.2	ELISA (IgG)	32.5 ± 27.7	NG	39/55
Tamura et al. [23]	1999	Japan	Case-control	52.2 ± 1.8	Urease test, histology, and culture	29.3 ± 5.4	20/41	26/48
Blusiewicz et al. [24]	2005	Poland	Case-control	50.8 ± 2.9	Urease test, histology	NG	19/30	22/31
Khedmat et al. [6]	2007	Iran	Case-control	47.9 ± 3.5	Urease test	46.9 ± 10.7	46/73	106/305
Khazaei et al. [25]	2008	Iran	Case-control	14.7 ± 3.4	Urease test, histology	HP(+) 22.5 ± 18.5 HP(-) 26.9 ± 32.5	16/24	5/25
Gioè et al. [26]	2008	Italy	Case-control	NG	RUT, Giemsa	NG	75/142	59/132
Asl and Nasri [27]	2009	Iran	Cross-sectional	56 ± 14	Giemsa	≥6	28/40	23/40
Sugimoto et al. [11]	2009	Japan	Case-control	58.8 ± 0.4	ELISA (IgG)	100.8 ± 3.6	NG	314/400
Chang et al. [28]	2010	Korea	Case-control	62 ± 9.8	RUT, histology	HP(+) 56.8 ± 26.9 HP(-) 66.4 ± 26.1	12/33	36/55

Ref.: reference; HD: hemodialysis; CAPD: continuous ambulatory peritoneal dialysis; NG: not given; RUT: rapid urease test.



(a)



(b)

FIGURE 2: (a) Prevalence of *H. pylori* in dialysis patients and controls with normal renal function. (b) The prevalence of *H. pylori* in hemodialysis patients and controls with normal renal function.

indeed showed a significantly lower rate of *H. pylori* infection ($P < 0.00001$) than those with normal renal function, while it is another story when it comes to those who endured dialysis duration shorter than four years [6, 14, 15, 21–23, 25] ($P = 0.27$) with no difference in *H. pylori* infection rate between two groups.

3.4. *Effect of H. pylori Status on Duration of Dialysis.* A few previous studies have shown that *H. pylori* positive patients required a significantly shorter course of dialysis

than uninfected patients [22, 31]. Among the included studies, five studies [4, 15, 21, 22, 25, 28] evaluated the relationship between *H. pylori* status and duration of dialysis. However, no statistical significance was observed between *H. pylori* negative and *H. pylori* positive patients. The weighted mean difference between these studies was 4.56 (95% CI: -1.55 – 10.67 , $P = 0.14$) (Figure 6).

3.5. *Endoscopic Findings.* We compared the endoscopic findings between the dialysis and control groups, mainly

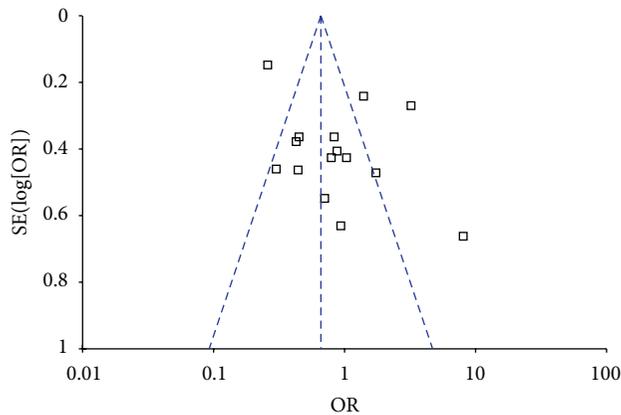


FIGURE 3: Funnel plot for 15 studies.

concerning gastritis and ulcerative diseases. Ten studies [4, 6, 10, 11, 13, 15, 21, 24, 26, 28] provided detailed endoscopic information regarding, for example, gastritis, ulcerative diseases, and intestinal metaplasia. The incidence of gastritis and ulcerative diseases in dialysis patients and normal controls was 66.2% versus 56.2% ($P = 0.99$) and 13.7% versus 24.9% ($P = 0.08$), respectively. There are no significant differences in endoscopic abnormalities between the dialysis patients and the controls with normal renal function (Figures 7(a) and 7(b)).

4. Discussion

Recently, more and more evidence has shown that *H. pylori* is related to extragastrointestinal diseases such as iron deficiency anemia, idiopathic thrombocytopenic purpura [32], and diabetes mellitus [33]. Moreover, patients with CRF usually suffer from systemic or local chronic circulatory failure (or both) [34], hypergastrinemia [21], high ammonia levels [35], and enhanced inflammation that facilitates *H. pylori* infection. In the present study, we performed a meta-analysis and found that CRF patients on dialysis treatment had an overall *H. pylori* infection rate of ~50.8%, which was relatively but not significantly lower than the 55.6% in controls ($P = 0.55$).

Upon investigating the association between *H. pylori* infection and the different types of dialysis, we found that *H. pylori* infection was not statistically associated with hemodialysis specifically. However, the *H. pylori* infection rate in the hemodialysis group (54.5%) was slightly higher than that of the control (45.9%), which contrasts with the results of the overall analysis. Due to lack of data, we were not able to analyze the difference in *H. pylori* prevalence between CRF patients undergoing CAPD and those receiving hemodialysis. Thus, our results from these studies revealed that the prevalence of *H. pylori* infection is similar between CRF patients who were receiving dialysis and the control group with normal renal function ($P > 0.05$).

From the results of the present study, it appears that CRF treatment with dialysis does not change the probability of *H. pylori* infection. Although one researcher went against current thought and concluded that the level of urea is not

a risk factor in *H. pylori* colonization [28], neither theory has proved definitive and more research is required to clarify the issue. Among the included studies in our meta-analysis, some researchers [4, 11, 13, 28] found that the prevalence of *H. pylori* in CRF patients undergoing dialysis was significantly lower than in non-CRF controls with or without gastrointestinal symptoms. The truth is that many CRF patients who receive dialysis inevitably have access to antibiotics, proton pump inhibitors, or H2 receptor antagonists which then influence the *H. pylori* infection rate to some extent [13, 36]. Moreover, gastric atrophy progresses along with decreased secretion of acid [37] as well as higher levels of proinflammatory cytokines [38] in CRF patients, making *H. pylori* difficult to survive. Apart from these, the prevalence of *H. pylori* varies widely across different demographic and geographic areas due to economic situations, sanitary conditions, cultural habits, and more.

Our subgroup analysis revealed that the prevalence of *H. pylori* of those patients who were on dialysis for longer than 4 years was significantly lower than of individuals with normal renal function, while the duration of dialysis between *H. pylori* negative and *H. pylori* positive patients did not differ from each other. It is in accordance with other studies. Sugimoto et al. [11] showed that the prevalence of *H. pylori* infection decreased in the first 4 years of dialysis and plateaued after 5 years of treatment and it was not affected by basement diseases. He and his colleagues concluded that more than one-third of patients who had received approximately four years of dialysis treatments had been naturally cured of *H. pylori* infections. Nakajima et al. [31] also reported that the prevalence of *H. pylori* decreased along with extended hemodialysis duration of two years and more. They declared that the reduction of *H. pylori* prevalence in long-term dialysis patients was due to reduction of gastric acid secretion related to chronic gastritis or frequent antibiotic consumption. Nevertheless there are actually conflicts about the relationship between *H. pylori* status and duration of dialysis. Several studies argued that duration of dialysis was inversely related to *H. pylori* colonization in dialysis patients [39–41], and some found an opposite result [42]. Yet, the underlying mechanism is still obscure. More investigations are warranted to be conducted to elucidate these findings in the future.

Endoscopic abnormalities such as erosive gastritis, duodenitis, and peptic ulcers are often found in CRF and dialysis patients. In some studies, peptic ulcers and gastroduodenal mucosa lesions were associated with *H. pylori* infection [43–47]. Khedmat et al. [6] showed that there was no significant difference in the rate of nonerosive gastritis, duodenitis, and gastric ulcer diseases between hemodialysis patients and those with normal renal function. These findings are in accordance with the results of our present meta-analysis, which indicated no statistical differences between dialysis patients and normal controls ($P > 0.05$) concerning endoscopic gastritis and ulcerative diseases. Thus it seems that dialysis itself is not a risk factor for the occurrence of gastritis or ulcers, although it is still necessary to perform endoscopy in dialysis patients with gastrointestinal symptoms. However, the above results rarely came from children's studies. Whether to recommend upper gastrointestinal examination based on

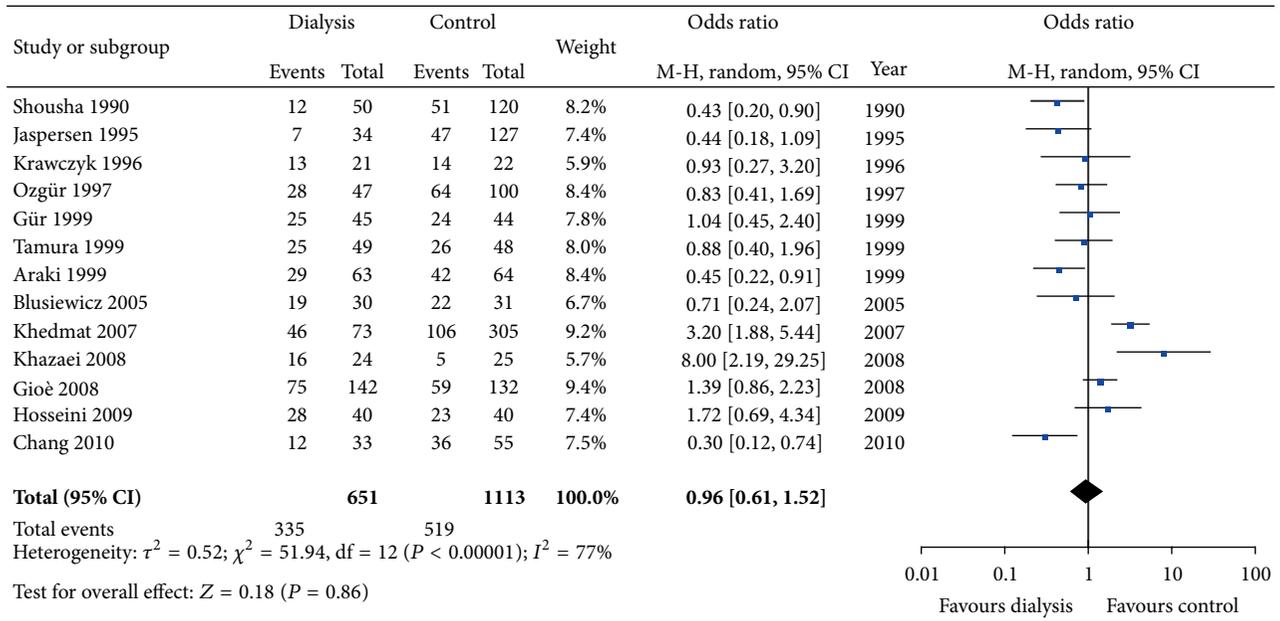
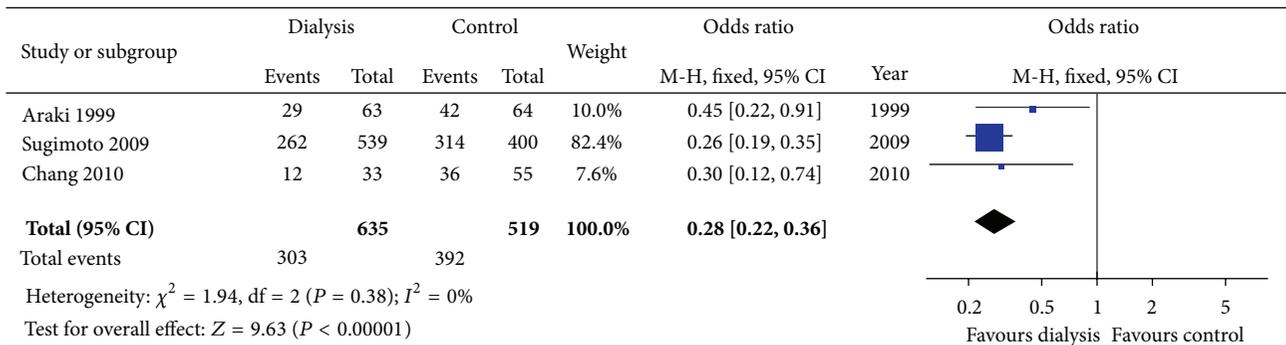
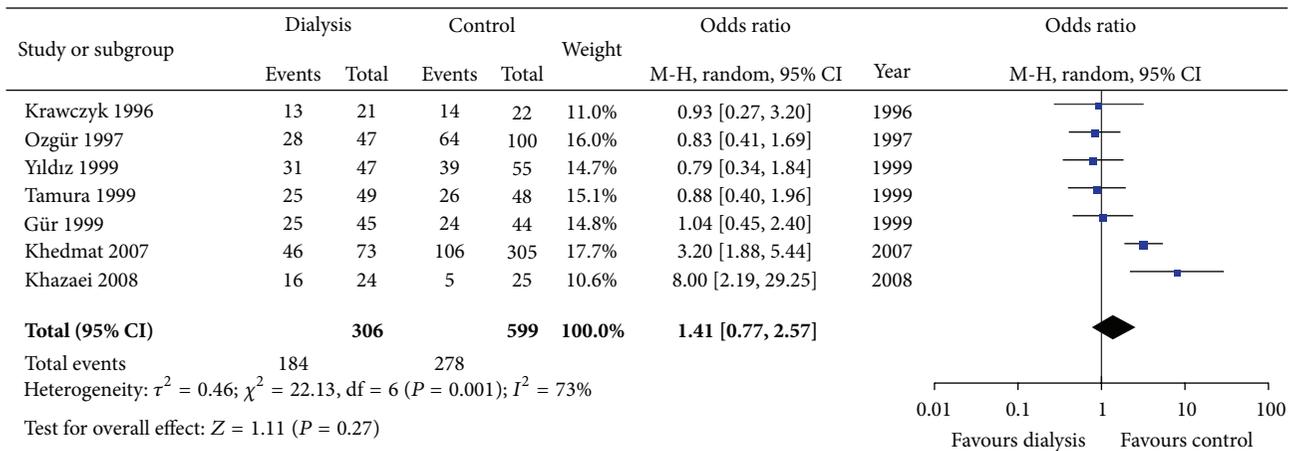


FIGURE 4: Various methods for detecting *H. pylori* infection (excluding IgG titer).



(a)



(b)

FIGURE 5: (a) Effect of dialysis duration (>4 years) on *H. pylori* prevalence. (b) Effect of dialysis duration (≤ 4 years) on *H. pylori* prevalence.

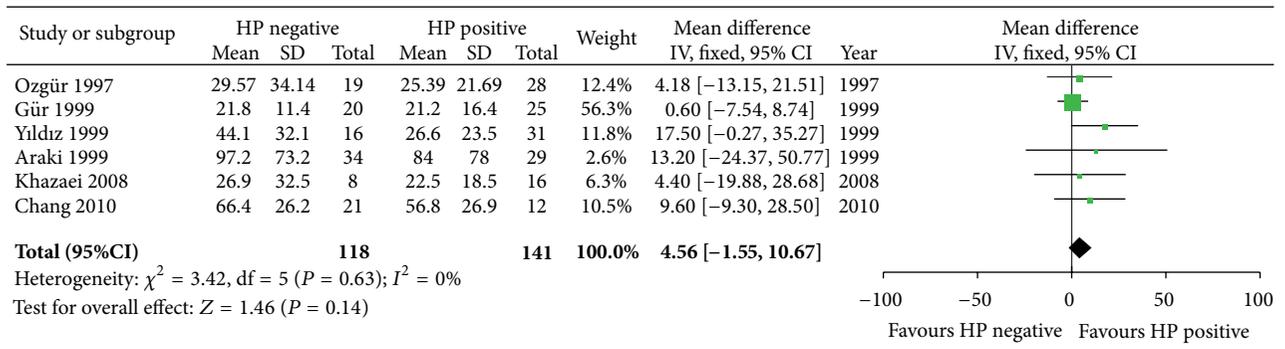
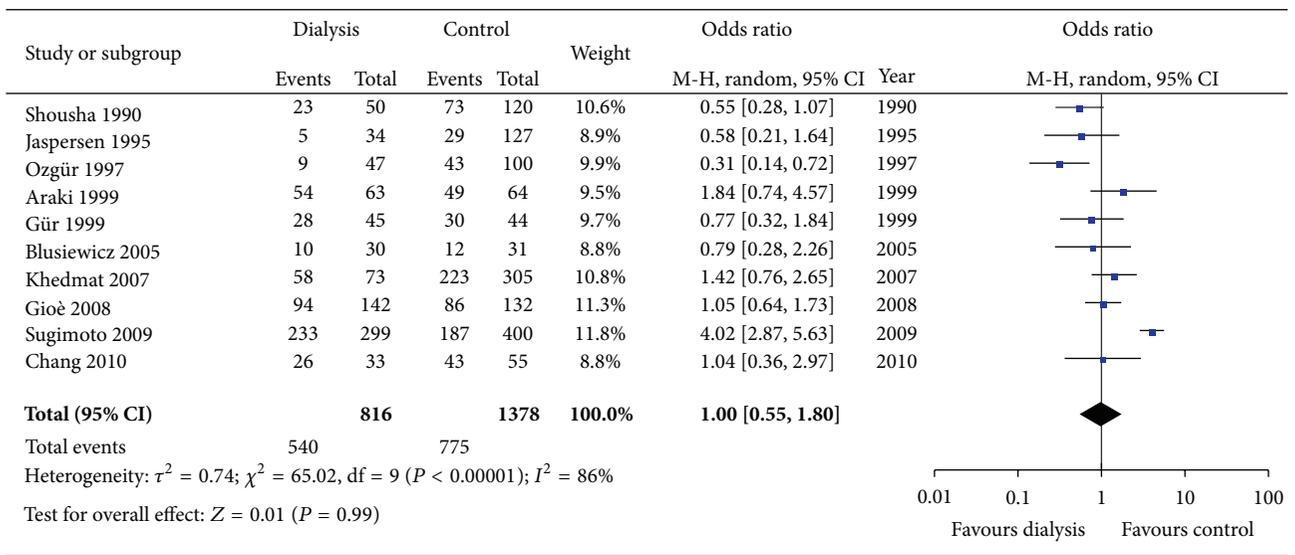
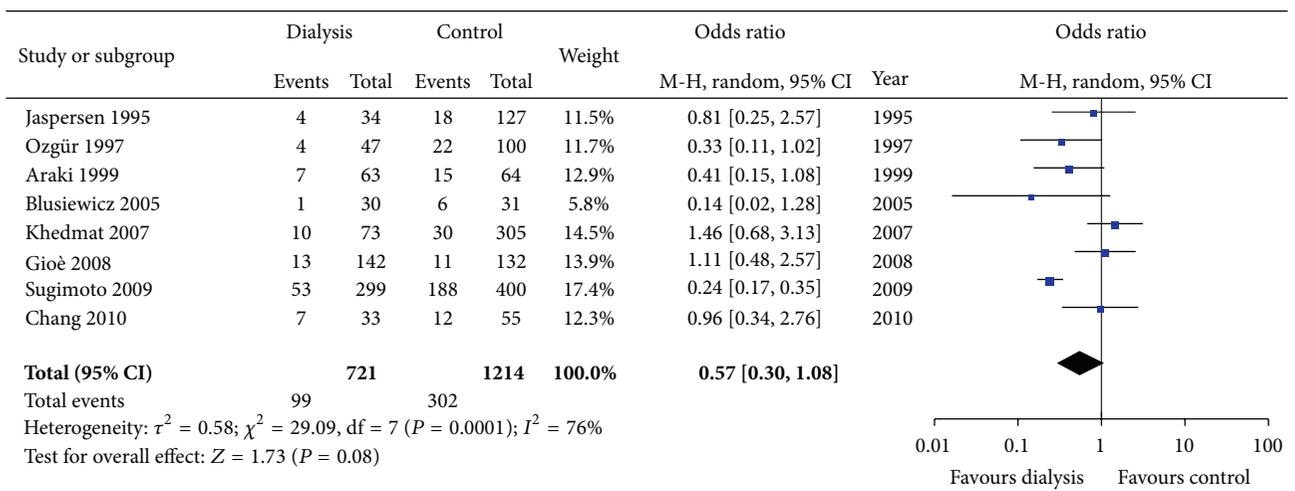


FIGURE 6: Association between *H. pylori* status and duration of dialysis in CRF patients.



(a)



(b)

FIGURE 7: (a) Incidence of gastritis between CRF patients on dialysis and normal controls. (b) Incidence of ulcer diseases between CRF patients on dialysis and normal controls.

symptoms requires more consideration in pediatric dialysis patients.

Although in the present meta-analysis we found no significant difference in *H. pylori* prevalence between dialysis patients and control subjects, according to some studies [21, 48, 49] successful eradication of *H. pylori* would lead to a significant decrease in dyspeptic symptoms, improvement in upper endoscopic results, and reduction in serum gastrin concentrations among hemodialysis patients. In such patients, due to impaired renal function and decline in the rate of excretion of drugs, the ideal treatment regimen should emphasize high efficacy and few adverse effects. Seyyedmajidi et al. conducted a randomized controlled trial comparing sequential therapy and standard triple therapy for *H. pylori* eradication in uraemic and nonuraemic patients. The eradication rates did not differ with both sequential and standard therapeutic regimens in the patients and normal controls. They preferred the standard triple therapy due to its lower side effects and complexity [50]. Chang et al. [28] found that a 7-day triple therapy with a low-dose OAC (omeprazole, amoxicillin, and clarithromycin) regimen was effective and safe for eradication of *H. pylori* infection in hemodialysis patients, with the consideration that amoxicillin and clarithromycin are primarily eliminated via the renal route. Further studies investigating the effect of eradication of *H. pylori* on symptom relief of dialysis patients are necessary.

When weighing the findings of the present meta-analysis, it is imperative to note that these studies were all case-control or cross-sectional studies, each performed at a single center with a cohort, and the sociodemographic characteristics of the populations were unclear. Although we adjusted for potential confounders, heterogeneity still existed among the study designs; confounding is an intrinsic limitation of these observational studies, so we precluded any assessment of causality in reported associations. Also, variables such as age and gender may be important considerations in the analysis of risk factors, but here we were unable to adjust for them, mainly due to incomplete data.

5. Conclusion

In the present meta-analysis there was no evidence of a significant association between infection with *H. pylori* and dialysis treatments for CRF patients. With heterogeneity limiting certainty of this association, there is a need for well-conducted randomized controlled trials to further verify these findings. According to subgroup analysis dialysis treatments for more than 4 year appears to have a protective effect against *H. pylori* infection; mechanistic studies of this negative association are needed to be further identified. It is indeterminate whether *H. pylori* status would affect duration of dialysis in CRF patients or whether endoscopic abnormalities of dialysis patients are related to *H. pylori* infection; further clinical studies investigating the effect of *H. pylori* infection on endoscopic findings of dialysis patients are necessary.

Conflict of Interests

The authors declare no conflict of interests.

Authors' Contribution

Min Gu and Shuping Xiao contributed equally to the work.

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Research Article

Ten-Day versus 14-Day Levofloxacin-Containing Triple Therapy for Second-Line Anti-*Helicobacter pylori* Eradication in Taiwan

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Second-line *Helicobacter pylori* (*H. pylori*) eradication with fluoroquinolone-containing triple therapy is one of the recommended treatment options, but neither 7-day nor 10-day regimens provide >90% success rates. The current retrospective study aimed to clarify the effects of 10-day and 14-day levofloxacin-containing triple therapies for second-line *H. pylori* eradication in a Taiwanese cohort and to evaluate the potential clinical factors influencing eradication. A total of 200 patients who failed *H. pylori* eradication using the standard triple therapy were prescribed with either a 10-day (EAL-10) or a 14-day (EAL-14) levofloxacin-containing triple therapy group (levofloxacin 500 mg once daily, amoxicillin 1 g twice daily, and esomeprazole 40 mg twice daily). Follow-up studies to assess treatment response were carried out 8 weeks later. Eradication rates attained by EAL-10 and EAL-14 were 75.6%; 95% CI = 63.9–85.3% and 92.5%; 95% CI = 84.5–98.1%, $P = 0.002$ in the per protocol analysis and 68%; 95% CI = 56.6–78.5% and 86%; 95% CI = 76.8–93.4%, $P = 0.002$ in the intention-to-treat analysis. The duration of *H. pylori* therapy is the independent risk factor of *H. pylori* eradication ($P = 0.003$). In conclusion, 14-day levofloxacin-containing triple therapy can provide a >90% *H. pylori* eradication rate, but 10-day treatment duration may be suboptimal. The longer duration of *H. pylori* therapy (14 days) is the independent risk factor.

1. Introduction

Many gastrointestinal diseases, either benign or malignant, are associated with *Helicobacter pylori* (*H. pylori*) infections, including peptic ulcer diseases, gastric adenocarcinoma, and gastric mucosa-associated lymphoid tissue lymphoma (MALToma) [1–3]. The successful rate of standard first-line triple therapies using a proton pump inhibitor (PPI), clarithromycin, and either amoxicillin or metronidazole for 7 to 14 days has dropped to less than 80% in many countries especially in areas of high clarithromycin resistance [4–7]. The Maastricht IV/Florence consensus report states that the standard treatment to eradicate *H. pylori* infection is triple therapy; using a proton pump inhibitor (PPI), clarithromycin, and either amoxicillin or metronidazole for 7 to 14 days

is recommended for first-line empirical treatment in areas of low clarithromycin resistance, while bismuth-containing quadruple therapy is also an alternative [8]. In areas of high clarithromycin resistance, bismuth-containing quadruple therapies, sequential treatment, or nonbismuth quadruple therapy is used for first-line empirical treatment.

Avoiding problems due to antibiotic resistance has become an important issue when deciding a second-line therapy for *H. pylori* infection [9–11]. Quinolone has the disadvantage of easily acquired drug resistances [3]. It is therefore an important issue to prescribe it wisely targeting at achieving a high eradication rate. A quinolone-containing triple therapy is recommended by both the Maastricht IV/Florence Consensus Report and the second Asia-Pacific Consensus

Guidelines [8, 12]. However, even large meta-analyses of second-line *H. pylori* eradication with fluoroquinolone-based triple therapy have shown that neither 7 days nor 10 days of therapy provide 90% or better treatment success [13]. Previous publications in Taiwan used 7-day levofloxacin-containing therapy and attained 75.3–80.3% of eradication rates [10, 12, 14, 15]. However, the reports on second-line eradication by using 14-day levofloxacin-containing triple therapy are few in the literature.

The current retrospective study aimed to clarify the effects of 10-day (EAL-10) and 14-day (EAL-14) esomeprazole/amoxicillin/levofloxacin therapy for patients who failed to have *H. pylori* eradicated after standard triple therapy in Taiwan and to determine the potential clinical factors influencing the eradication.

2. Materials and Methods

2.1. Patients. A total of 200 *H. pylori*-infected patients who failed *H. pylori* eradication using the standard triple therapy (PPI twice daily, 500 mg of clarithromycin twice daily, and 1 g of amoxicillin twice daily) for 7 days were recruited. All the patients were at least 18 years of age and had received endoscopic exam which showed peptic ulcers disease or gastritis. The confirmations of *H. pylori* eradication failure were defined as positive results for both the rapid urease test and histology after first-line eradication. The criteria for exclusion were (a) ingestion of antibiotics, bismuth, or PPI within 4 weeks, (b) allergic history to the medications used, (c) previous gastric surgery, (d) the coexistence of serious concomitant illness (e.g., decompensated liver cirrhosis and uremia), and (e) pregnancy. These 200 patients were prescribed with either a 10-day levofloxacin-containing triple therapy group (levofloxacin 500 mg once daily, amoxicillin 1 g twice daily, and esomeprazole 40 mg twice daily for 10 days, EAL-10) or a 14-day levofloxacin-containing triple therapy group (EAL-14). Patients were followed up to assess the adverse effects and drug compliance after they finished the medications. All patients received either an endoscopy or a urea breath test eight weeks later. Besides, we also performed a backup urea breath test on all participants to avoid any false-negative results. The definition of poor compliance was that the patient failed to finish 80% of all medications due to adverse effects [5, 15].

This study was approved by both the Institutional Review Board and the Ethics Committee of Chang Gung Memorial Hospital (IRB102-0921B). All patients provided their written informed consent before endoscopic interventions.

2.2. Outcomes. The primary endpoint was the successful eradication of *H. pylori*. There was additional analysis of adverse events during therapy.

2.3. Diagnosis of *Helicobacter pylori* Infection

2.3.1. Rapid Urease Test. The rapid urease test involved the collection of gastric antrum biopsy specimens by endoscopy, which were tested using a urea agar base enriched with 40%

urea solution (eUAB, Oxoid) and a commercial rapid urease test (Pronto Dry, Medical Instrument Corp, Switzerland) [16]. The results of the rapid urease test were interpreted as positive if the color of the gel turned pink or red when examined after 1 h at room temperature.

2.3.2. Urea Breath Test. The urea breath test was performed according to our previous studies [17]. The cut-off value was set at 4.8% of $\delta^{13}\text{CO}_2$. Staffs were blinded to the *Helicobacter pylori* status performed to the test.

2.4. Statistical Analysis. The primary outcome variables were the rates of eradication, the presence of adverse events, and the level of patient compliance. Using the SPSS program (Statistical Package for the Social Sciences version 18, Chicago, IL, USA), the chi-square test with or without Yates's correction for continuity and Fisher's exact test were used to compare the major outcomes between groups. Eradication rates were analyzed by both the intention-to-treat (ITT) and per protocol (PP) approach. ITT analysis included all assigned patients who had taken at least one dose of the study medication. Patients whose infection status was unknown following treatment were considered treatment failures for the purposes of the ITT analysis. The PP analysis excluded patients with unknown *H. pylori* status following therapy and those with major protocol violations. A *P* value of less than 0.05 was considered statistically significant. To determine the independent factors that affected the treatment response, clinical parameters were analyzed by univariate and multivariate analyses.

3. Results

A total of 200 patients were enrolled (100 each in the EAL-10 and the EAL-14 group). Ten patients lost to follow-up in EAL-10 group and 7 in the EAL-14 group resulted in 90 in the PP study for EAL-10 and 93 for EAL-14 (Figure 1). The demographic data of the two groups are summarized in Table 1, and none of the variables showed significant difference between EAL-10 and EAL-14 groups.

Eradication rates attained by EAL-10 and EAL-14 were 75.6%; 95% CI = 63.9–85.3% and 92.5%; 95% CI = 84.5–98.1%, *P* = 0.002 in the PP analysis and 68%; 95% CI = 56.6–78.5% and 86%; 95% CI = 76.8–93.4%, *P* = 0.002 in the ITT analysis (Table 2).

3.1. Adverse Events and Complications. The adverse events were 11% (11/100) in EAL-10 group and 16% (16/100) in EAL-14 group (Table 2). These adverse events were abdominal pain, constipation, diarrhea, dizziness, headache, nausea/vomiting, and skin rash, but they were mild and had little disturbance in patients' daily activities (Table 3). Both groups had good drug compliances (100% in EAL-10 group versus 99% in EAL-14 group).

3.2. Factors Influencing the Efficacy of the Anti-*H. pylori* Therapies. Univariate analysis showed that the duration of *H. pylori* eradication (*P* = 0.002) was the clinical factor

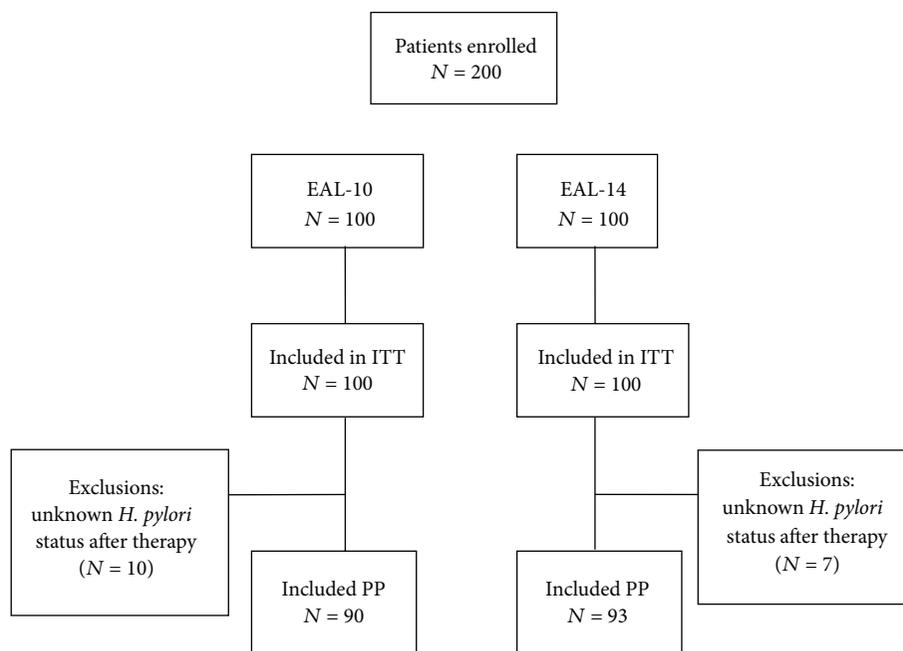


FIGURE 1: Disposition of patients.

TABLE 1: Demographic data and endoscopic appearances of the two patient groups.

	EAL-10 (n = 100)	EAL-14 (n = 100)
Characteristics		
Age (year) (mean ± SD)	55.6 ± 13.2	57.6 ± 12.8
Gender (male/female)	44/56	45/55
Smoking	9 (9%)	6 (6%)
Alcohol consumption	8 (8%)	13 (13%)
Previous history of peptic ulcer	21 (21%)	29 (29%)
Endoscopic findings		
Gastric ulcer	36 (36%)	35 (35%)
Duodenal ulcer	18 (18%)	20 (20%)
Gastric and duodenal ulcer	11 (11%)	14 (14%)
Unspecified (include gastritis)	35 (35%)	31 (31%)

TABLE 2: The major outcomes of EAL-10 and EAL-14 eradication therapy.

	EAL-10 (n = 100)	Eradication rate	
		EAL-14 (n = 100)	P value
Intention-to-treat	68% (68/100)	86% (86/100)	0.002
Per-protocol	75.6% (68/90)	92.5% (86/93)	0.002
Adverse event	11% (11/100)	16% (16/100)	0.301
Compliance	100% (100/100)	99% (99/100)	1.000

EAL-10: esomeprazole/amoxicillin/levofloxacin triple therapy × 10 days and EAL-14: esomeprazole/amoxicillin/levofloxacin triple therapy × 14 days.

influencing the efficacy of *H. pylori* eradication therapy (Table 4). Simultaneously, multivariate analysis showed the duration of *H. pylori* eradication (EAL-10 versus EAL-14, OR: 3.98, 95% CI: 1.60–9.84, $P = 0.003$) was the independent risk factor of successful *H. pylori* eradication (Table 5).

4. Discussion

Quinolone-containing triple therapy is one of the recommended second-line therapies after the failure of the standard first-line empirical clarithromycin-containing therapy, with bismuth-containing quadruple therapy as an alternate [8].

TABLE 3: Adverse events during EAL-10 and EAL-14 therapies.

Adverse events	EAL-10 (<i>n</i> = 100)	EAL-14 (<i>n</i> = 100)	<i>P</i> value
Abdominal pain	5	7	0.552
Constipation	2	2	1.000
Diarrhoea	0	4	0.121
Dizziness	4	1	0.369
Headache	6	3	0.498
Nausea/vomiting	2	2	1.000
Skin rash	0	2	0.497

EAL-10: esomeprazole/amoxicillin/levofloxacin triple therapy × 10 days and EAL-14: esomeprazole/amoxicillin/levofloxacin triple therapy × 14 days.

TABLE 4: Univariate analysis of the clinical factors influencing the efficacy of *Helicobacter pylori* eradication.

Principle parameter	Case no.	Eradication rate	<i>P</i> value
Age			
<60 years	111	82.0% (91)	0.318
≥60 years	72	87.5% (63)	
Sex			
Female	98	81.6% (80)	0.316
Male	85	87.1% (74)	
Smoking			
(-)	168	83.3% (140)	0.472
(+)	15	93.3% (14)	
Previous history of peptic ulcer			
(-)	138	81.9% (113)	0.141
(+)	45	91.1% (41)	
HP eradication (per protocol)			
EAL-10	90	75.6% (68)	0.002
EAL-14	93	92.5% (86)	
Compliance			
Good	183	84.2% (154)	—
Poor	0	0% (0)	

EAL-10: esomeprazole/amoxicillin/levofloxacin triple therapy × 10 days and EAL-14: esomeprazole/amoxicillin/levofloxacin triple therapy × 14 days.

However, we are also aware that bismuth salts are not available in many hospitals. As a matter of fact, such triple therapy with quinolone-containing regimens has been shown to be a good alternative treatment as a second-line *H. pylori* therapy with comparable results to the recommended bismuth-based quadruple therapy [18–20]. Large meta-analyses of second-line *H. pylori* eradication with fluoroquinolone triple therapy have shown that 7 to 10 days of therapy could not provide 90% or better treatment success [14, 21]. Published papers on the efficacies of 14-day quinolone-containing triple therapy for second-line therapy are very few, and none of them offered head-to-head data on efficacies for EAL-10 and EAL-14 therapies in similar cohort. This is a very important message because one must target to eradicate the bacteria with a better formula to avoid subsequent quinolone resistance if the eradication failed.

Levofloxacin is a levorotatory isomer of ofloxacin with known activity against many Gram-negative and Gram-positive bacteria [22]. The mode of action of levofloxacin is based on the inhibition of bacterial DNA topoisomerase II. A levofloxacin-containing triple therapy is simple and well tolerated and has high compliance (100% and 99% in the current study). The relatively low incidence of adverse events among the EAL group was the key factor related to this good compliance. This is important because compliance plays a cardinal role in eradication. In addition, *in vitro* levofloxacin retains its activity even in dual *H. pylori* resistant strains to clarithromycin and metronidazole [23, 24]. Similar effects have been observed *in vivo*, showing that most of the dual metronidazole and clarithromycin resistances in *H. pylori* infections are cured with the levofloxacin-containing regimen [18, 25]. Moreover, there is an *in vivo* synergistic effect of the quinolone antimicrobial agent and the proton-pump inhibitor on strains of *H. pylori* [26].

Drug resistance to antibiotics is an important key factor in successful *H. pylori* eradication. Interestingly, it was just about a decade ago that levofloxacin was chosen as the most promising agent used to overcome antimicrobial resistance among the new antibiotic and drug combinations that had been evaluated, including fluoroquinolones, rifabutin, furazolidone, and azithromycin [27]. Gisbert et al. reported that levofloxacin triple scheme was superior to quadruple therapy (81% versus 70%) with a lower incidence of side effects (19% versus 44%). Again, the 10-day levofloxacin-based triple scheme was superior to the same 7-day therapy (81% versus 73%) [13]. Today, quinolone resistance is becoming a major concern for the EAL therapy. Just like metronidazole and clarithromycin, drug resistance to levofloxacin is becoming an important factor responsible for unfavorable results. In Taiwan, Kuo et al. reported that levofloxacin-resistant strain was found in 28.3% of patients [11]. In fact, primary levofloxacin resistance has been increasing in most parts of the world with values of 5.5% to 32.3% in countries such as Japan, Brazil, Italy, Hong Kong, and Republic of Korea [28–32]. Therefore, it is very important that the use of quinolone-containing triple therapies need cautious monitoring, because Taiwan is an endemic area for tuberculosis infection [33].

Another reason for the failure of quinolone-containing triple therapies as second-line eradication regimens is the duration of the treatment instead of the dosage. Both the univariate and multivariate analyses in the current study showed that the length of *H. pylori* treatment was the clinical factor influencing the efficacy of eradication. This was similar to Caro and colleagues' report that the duration of treatment is the crucial factor influencing eradication rate but not dosage [20]. In the systemic review reported by Gisbert et al, higher *H. pylori* cure rates with a 10-day rather than a 7-day regimen were found with the levofloxacin-amoxicillin-PPI combination (80% versus 68%), suggesting that the longer (10-day) therapeutic scheme should be chosen for levofloxacin-containing triple therapy [13]. In Taiwan, Cheng et al. also reported that prescribing 500 mg and 1000 mg levofloxacin per day did not affect the eradication rates [14]. Liou et al. also attained only 76.9% eradication with levofloxacin 750 mg once daily [34]. In both studies, the length of treatment

TABLE 5: Multivariate analysis of the clinical factors influencing the efficacy of *Helicobacter pylori* eradication.

Clinical factor	Coefficient	Standard error	Odds ratio (95% CI)	P value
Duration of <i>H. pylori</i> eradication (10 days versus 14 days)	1.38	0.46	3.98 (1.60–9.86)	0.003

EAL-10: esomeprazole/amoxicillin/levofloxacin triple therapy × 10 days and EAL-14: esomeprazole/amoxicillin/levofloxacin triple therapy × 14 days.

was only 7 days. The current study showed that EAL-10 could be suboptimal and only attained 75.6% eradication rate in the PP analysis. None of these studies with 7–10 days of levofloxacin-containing triple therapies attained 90% or better treatment success. Two recently published studies reported that extending the length of quinolone-containing triple therapies to 14 days could achieve eradication success up to 95% (moxifloxacin) and 93.6% (levofloxacin) [35, 36]. Consequently, the current study achieved an eradication rate of 92.5% in the EAL-14 group in PP analysis but only 75.6% in the EAL-10 group. Our study result adds on a potentially important message that 14 days should be the optimal length of treatment for quinolone-containing triple therapies as a second-line *H. pylori* treatment option instead of the 7–10-day regimens. The bottom line is that quinolone resistance is carefully monitored.

However, the current study encountered its limitations. First, since our laboratory could not perform CYP2C19 genotyping, we used an esomeprazole-based regimen because it had minimal first-pass metabolism and had a greater gastric acid suppression effect than omeprazole. Second, there was the lack of information regarding the prevalence of antimicrobial resistance.

5. Conclusions

A 14-day levofloxacin-containing triple therapy can provide a >90% *H. Pylori* eradication rate, but 10-day treatment duration may be suboptimal. The longer duration is the independent risk factor for eradication. This is a very important message since quinolone easily acquires resistance. Meanwhile, a continuous search for novel second-line therapeutic approaches which are cost-effective and minimize drug resistance to cure *H. pylori* infection is still mandatory.

Conflict of Interests

The authors declare that they received no current external funding sources for this study.

Authors' Contribution

Wei-Chen Tai and Chien-Hua Chiu contributed equally to the work.

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Research Article

Risk of Atherosclerosis and *Helicobacter pylori* Infection according to CD14 Promotor Polymorphism in Healthy Korean Population

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Background and Aim. We aim to elucidate the association of risk factors for atherosclerosis and *H. pylori* infection according to the promotor polymorphism of the CD14 gene in healthy Korean population. **Methods.** The patients who visited our hospital for routine health examinations and 266 healthy adults (170 males and 96 females) were enrolled in this study. The promotor polymorphism at -159C/T of the CD14 gene was determined by PCR-restriction fragment length polymorphism analysis. According to genetic polymorphism and *H. pylori* infection, we analyzed the risk of atherosclerosis. **Results.** The genotype frequencies were CC 7.9%, CT 45.1%, and TT 47.0%, respectively. There were no differences between specific genotypes of CD14 gene and *H. pylori* infection rate. As for HDL cholesterol level, there were significant differences among the three genotypes ($P < 0.01$). In subjects with *H. pylori* infection, no significant differences were observed between specific genotypes of CD14 gene and the risk factors of atherosclerosis. **Conclusion.** The promotor polymorphism at -159C/T of the CD14 gene was associated with the risk factor of atherosclerosis in healthy Korean population. However, it was not associated with the rate of *H. pylori* infection and *H. pylori* induced atherosclerotic risk.

1. Introduction

Clusters of differentiation 14 (CD14) receptor is a mediator of the inflammatory response by recognition of lipopolysaccharide (LPS), a major component of the outer cell wall of Gram-negative bacteria [1]. By inducing inflammatory reactions, chronic infection, such as *Helicobacter pylori* (*H. pylori*), plays an important role in the development of atherosclerosis and its associated complications [2–5]. Recently, it has been widely accepted that inflammation and infection play a key role in atherosclerosis and cardiovascular diseases [6–10]. Clinically, as with *H. pylori* infection, atherosclerotic cardiovascular abnormalities are common, and the infection of this bacterium may be introduced as a risk factor of coronary arterial disease (CAD), independent of known risk factors [2–5].

Moreover, genetic factors for the development of atherosclerosis would be associated with the LPS-mediated activation processes of monocytes and their receptor CD14 [11–13].

The CD14 gene promoter contains a single nucleotide polymorphism, and cytosine (C) to thymine (T) transition polymorphism at position -159 may influence the expression of CD14 [14]. C-to-T transition polymorphism at position -159 in the promoter region of the CD14 gene may affect the affinity of specificity protein (Sp protein) binding and modify transcriptional activity. This variation is important for the pathogenesis of inflammatory disease [14, 15]. It has been thought that a functional polymorphism in the promoter of the CD14 gene (CD14 -159C/T) is associated with *H. pylori*-related gastric carcinoma [16, 17], ischemic heart disease, and atherosclerosis [18–21]. Until now, several studies have

yielded conflicting results [22–24]. Because gastric carcinoma and cardiovascular disease are multifactorial disorders caused by the interaction of gene-by-gene and/or gene-by-environment interactions, it is hard to conclude the causal relationship between the specific genotype and the disease. Moreover, in Korean population, there are limited data available on the association of CD14 polymorphism and the specific diseases.

In this study, we focus on normal healthy Korean population and aim to elucidate the association of risk factors for atherosclerosis and *H. pylori* infection according to the promoter polymorphism of the CD14 gene.

2. Material and Methods

2.1. The Study Population. Healthy patients were enrolled from a teaching hospital of the Catholic University of Medicine, St. Vincent's Hospital, from March 2009 to February 2010. They were asymptomatic examinees of regular health screening with a simple symptom questionnaire at the Health Promotion Center of the same hospital and had endoscopic examinations for free nationwide gastric cancer screening in a Korean adult population. They had no history of hypertension, diabetes, cardiac disease, or any other chronic illness. Individuals with conditions that might have substantial effects on our study results (e.g., serum creatinine >2.5 mg/dL, total bilirubin >3.0 mg/dL), pregnant women, patients with psychiatric diseases, and patients who did not sign a consent form were excluded. Individuals with grossly severe gastric atrophy or ulceration or who were taking specific gastrointestinal medication, NSAIDs, or any other drugs were also excluded. Alcohol drinking was defined as consumption of at least 20 g alcohol/day and up to three times/week. Smoking was defined as current smoker.

2.2. Physical Examination and Collection of Specimen. Height, weight, and body mass indices (BMI, kg/m²) were measured using the Inbody 720 (Body Composition Analysis, Biospace Co.). The waist circumference was measured with the individual standing upright, with their top raised and under an exhalation state, at the middle area between the lowest area of the number 12 costa and the highest iliac crest, by an experienced person using a ruler with units of 0.1 cm. For systolic and diastolic blood pressure measurements, the subjects took sufficient rest while sitting down over a 5-minute period, and they were measured once with an automatic hemodynamometer.

After fasting for more than 12 hours, serum samples were collected from each patient's upper arm. Fasting blood sugar (FBS), aspartate transaminase (AST), alanine aminotransferase (ALT), total cholesterol, triglyceride, HDL cholesterol, high-sensitivity CRP (hs-CRP), lipoprotein a (Lp (a)), free T₄, thyroid stimulating hormone (TSH), and ferritin level were measured. Blood samples (10 mL) were obtained from all subjects and collected in a test tube containing EDTA or heparin.

In enrolled patients, two biopsy specimens were taken during upper gastrointestinal endoscopy from greater curvature side of the midantrum and corpus for histology. The diagnosis of *H. pylori* infection was made by the histologic evidence with a Warthin-Starry silver stain in any of two specimens from antrum and corpus.

3. Genetic Polymorphism

Genomic DNA was acquired from buffy-coat leukocytes using the AccuPrep Genomic DNA Extraction Kit (BIONEER CORPORATION, Daejeon, Republic of Korea). The promoter polymorphism at -159 of CD14 gene was evaluated using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. The PCRs were set up using i-Star Taq DNA polymerase (iNtRON BIOTECHNOLOGY, Seoul, Republic of Korea). In brief, we amplified the CD14 gene promoter region using the forward primer 5'-ATCATCCTTTTCCCACACC-3' and the reverse primer 5'-AACTCTTCGGCTGCCTCT-3'. In a total reaction volume of 10 μ L, 1 μ L 10X buffer, 10 pmol of each primer, 1 μ L of genomic DNA, and 1 μ L of dNTP mixture were combined. The following reaction conditions were established: an initial denaturation at 95°C for 5 minutes followed by 35 cycles at 95°C for 30 seconds, at 64°C for 30 seconds, and at 72°C for 30 seconds. The final extension step was carried out for 5 minutes at 72°C. The reactions were run on a Bio-Rad MyCycler thermal cycler (BIORAD, USA). 3 μ L of the resultant PCR products were digested overnight with HaeIII, the appropriate restriction enzyme (New England BioLabs, Beverly, MA, USA), and the digests were electrophoresed in 3% agarose gel. The CD14 C allele was cut into 2 fragments of 140 and 155 base pairs, whereas the T allele remained uncut, with a length of 295 base pairs.

4. Statistics

Statistical analysis was conducted using SPSS version 16.0 software. Data was expressed as means \pm standard deviation. Allele and genotype frequencies were compared via chi-squared tests. The normality in the distributions was assessed using normal probability plots. ANOVA or Student's *t*-test was used to assess normally distributed variables where appropriate. Kruskal-Wallis H test was used for analysis of factors that were not normally distributed. Differences at the level of $P < 0.05$ were regarded as statistically significant.

5. Ethics Statement

Informed consent was obtained from all patients, and the study was approved by the Institutional Review Board of the Catholic University of Korea (VC08TISI0082).

6. Results

6.1. Basal Characteristics of the Enrolled Patients. A total of 266 healthy adults (170 males and 96 females) were enrolled in this study. The mean age of the subjects was 47.99 ± 10.86 years, and no differences between males and females were

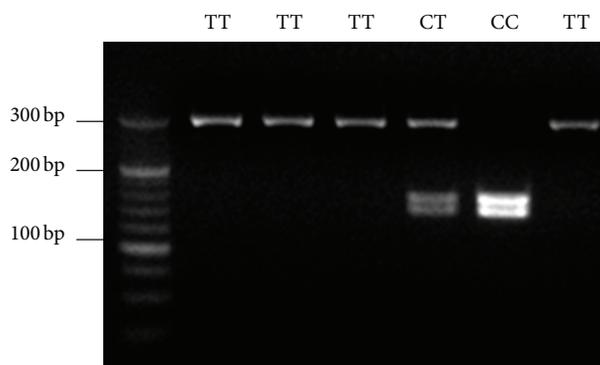


FIGURE 1: Detection of the $-159C/T$ polymorphism of CD14 gene by using PCR-RFLP. Digestion of the PCR products yielded bands of 295 bp in TT homozygotes, 140 and 155 bp in CC homozygotes, and all 3 bands (140, 155, and 295 bp) in heterozygotes.

detected (male: 47.60 ± 10.49 ; female: 48.69 ± 11.52 ; $P = 0.44$). The average BMI of the subjects was $24.33 \pm 2.92 \text{ kg/m}^2$, and the average waist circumference was $88.26 \pm 8.24 \text{ cm}$.

6.2. Analysis of Risk Factors for Atherosclerosis according to CD14 Polymorphism. Digestion of the PCR products for the promoter polymorphism at $-159C/T$ of the CD14 gene yielded bands of 295 bp in TT homozygotes, 140 and 155 bp in CC homozygotes, and all 3 bands (140, 155, and 295 bp) in heterozygotes (Figure 1). The genotype frequencies were CC 7.9%, CT 45.1%, and TT 47.0%, respectively. There were no differences in age, sex, the ratio of smokers, and alcohol drinkers between the three genotypes.

Between specific genotypes of CD14 gene and the risk factors of atherosclerosis, BMI, waist circumference, systolic blood pressure, diastolic blood pressure, fasting blood sugar, total cholesterol, hs-CRP, and Lp (a), no significant differences were observed. Also, no significant differences of liver function test, white blood cell, thyroid hormone, thyroid-stimulating hormone, and ferritin level existed according to the specific genotypes. However, as for HDL cholesterol level, there were significant differences among the three genotypes ($P < 0.01$ by ANOVA) (Table 1). When post hoc analysis was conducted, CC genotype was clearly associated with higher HDL cholesterol and lower TG level ($P = 0.04$), compared with CT and TT genotypes.

6.3. Analysis by Gender. When the subjects were divided by gender, the risk of atherosclerosis according to CD14 polymorphisms was evaluated. In male, there were no differences between specific genotypes of CD14 gene and the risk factors of atherosclerosis (Table 2). In female, no relevant associations were observed except HDL cholesterol and TSH level ($P = 0.05$) (Table 3).

6.4. *H. pylori* Infection State and Analysis of Risk Factors for Atherosclerosis in Individuals with *H. pylori* Infection. Total of

133 subjects (50.0%) had associated *H. pylori* infection. There were no significant differences of atherosclerosis risk between *H. pylori*-positive and *H. pylori*-negative subjects.

The frequencies of the CC, CT, and TT genotype with *H. pylori* infection were 10.5% (14/133), 48.9% (65/133), and 40.6% (54/133), and T allele frequency in subjects with *H. pylori* infection was 0.70. No significant difference between specific CD14 genotype and *H. pylori* infection status was found ($P = 0.06$). In subjects with *H. pylori* infection, no significant differences were observed between specific genotypes of CD14 gene and the risk factors of atherosclerosis (Table 4).

7. Discussion

Chronic *H. pylori* colonization may be associated with an increased risk for atherosclerosis [25, 26] and plays a causative role in the autoimmune diseases [27]. But the mechanisms by which this bacterium may trigger atherosclerosis remain incompletely understood. The underlying hypothesis is that it has atherogenic capacities by chronic low-grade activation of the hemostasis cascade. CD14 as a pattern recognition molecule of the innate immune response is the main receptor for LPS generated by Gram-negative bacteria such as *H. pylori*. It stimulates the release of proinflammatory cytokines, which is involved in the induction of the synthesis of tumor necrosis factor- α (TNF- α), interleukin-1 (IL-1), interleukin-6 (IL-6), growth factors, coagulation factors, and primary immune response. Activated macrophages via CD14 receptor may induce IL-6 production within the atheroma. An abnormally high plasma level of IL-6 represents a further risk factor for plaque rupture and atherosclerosis progression [10, 11, 28, 29].

Previously, it has been reported that acute myocardial infarction/atherosclerosis are more closely related to CD14 $-159TT$ homozygotes [30–32]. Meta-analysis reported that the causal relationship between TT genotype and ischemic heart disease was probably established only in the East Asian population, but there was no significant association in a European population and an Indian population [33]. In our study, 47.0% of the participants evidenced the TT genotype, which is substantially higher than the 15.6–27.4 % reported in studies conducted in other countries [14–16, 18, 19, 22]. It suggested that the contribution of genetic determinants might differ significantly between ethnicities. For the most part, the ischemic heart disease has complex origins that are caused by a combination of genetic, environmental, and lifestyle factors. Therefore, it is hard to conclude that the single gene variants will be proven as probable association with the specific disease. In this study, we focused on the relation between a risk factor and genetic polymorphism, and selected healthy adults without specific gastrointestinal symptoms to reduce the risk of selection bias. Our data showed that $-159CT$ and TT genotypes of CD14 gene have lower HDL cholesterol and higher triglyceride serum concentrations as compared to CC genotype. As demonstrated previously, low HDL cholesterol was well known as a potent risk factor even in the presence of very low level of LDL cholesterol [34]. Evidence of the association between triglyceride values and

TABLE 1: Risk factors of atherosclerosis according to CD 14 –159 genotypes.

	CC	CT	TT	P*
Age	47.00 ± 11.95	47.56 ± 11.43	48.44 ± 10.04	0.75
Sex (male : female)	11 : 10	80 : 40	79 : 46	0.44
Smoking	12	50	42	0.09
Alcohol drinking	14	72	68	0.47
<i>H. pylori</i> infection	14	65	54	0.06
Waist circumference (cm)	87.52 ± 7.97	88.88 ± 8.65	87.79 ± 7.93	0.58
Body mass index (Kg/m ²)	24.11 ± 2.83	24.34 ± 2.99	24.37 ± 2.91	0.94
SBP (mmHg)	125.28 ± 18.59	126.63 ± 13.52	127.07 ± 14.17	0.88
DBP (mmHg)	75.44 ± 12.76	75.32 ± 9.10	76.23 ± 9.87	0.78
Hemoglobin (g/dL)	14.37 ± 1.35	14.68 ± 1.88	14.59 ± 1.60	0.72
ESR	15.27 ± 1.15	12.84 ± 1.04	13.70 ± 1.03	0.76
AST (IU/L)	24.90 ± 9.12	24.54 ± 9.74	23.05 ± 7.06	0.33
ALT (IU/L)	24.07 ± 15.27	28.36 ± 19.11	26.14 ± 14.12	0.41
rGTP (IU/L)	30.81 ± 31.82	42.44 ± 47.29	36.10 ± 33.00	0.31
FBS (mg/dL)	96.24 ± 11.49	102.22 ± 21.71	103.01 ± 25.21	0.45
Uric acid (mg/dL)	5.43 ± 1.34	5.57 ± 1.54	5.33 ± 1.45	0.48
Triglycerides (mg/dL)	105.38 ± 48.85 [†]	159.04 ± 123.83	160.76 ± 97.53	0.08
Total cholesterol (mg/dL)	191.52 ± 31.01	204.02 ± 39.22	194.77 ± 32.89	0.13
HDL cholesterol (mg/dL)	52.67 ± 15.04 [†]	45.87 ± 9.61	44.10 ± 9.19	<0.01*
hs-CRP (mg/dL)	0.23 ± 0.23	0.19 ± 0.40	0.21 ± 0.40	0.92
Lp (a)	11.93 ± 9.92	11.74 ± 13.09	15.92 ± 17.52	0.35
Free T4 (ng/dL)	0.98 ± 0.17	1.03 ± 0.15	1.16 ± 0.91	0.23
TSH (μg/mL)	2.14 ± 1.79	1.89 ± 1.97	1.70 ± 2.68	0.20
Ferritin (ng/mL)	133.00 ± 164.03	134.39 ± 88.13	145.43 ± 129.98	0.87

All values are presented as mean ± standard deviation of the mean. *P values were obtained by one-way ANOVA. [†]P values (CC genotype versus CT/TT genotypes) were obtained by Scheffé's post hoc test after one-way ANOVA ($P < 0.05$).

H. pylori: *Helicobacter pylori*, SBP: systolic blood pressure, DBP: diastolic blood pressure, ESR: erythrocyte sedimentation rate, AST: aspartate transaminase, ALT: alanine aminotransferase, FBS: fasting blood sugar, hs-CRP: high-sensitivity C-reactive protein, Lp (a): lipoprotein a, and TSH: thyroid stimulating hormone.

TABLE 2: Risk factors for atherosclerosis according to male gender.

	CC	CT	TT	P*
Age	44.18 ± 7.25	47.14 ± 11.35	48.77 ± 9.97	0.29
Waist circumference (cm)	86.68 ± 8.73	89.63 ± 8.41	88.75 ± 7.20	0.64
Body mass index (Kg/m ²)	25.28 ± 2.82	24.73 ± 2.94	24.85 ± 2.62	0.82
SBP (mmHg)	131.00 ± 15.61	128.19 ± 12.39	129.19 ± 13.09	0.67
DBP (mmHg)	79.20 ± 13.27	76.18 ± 9.13	78.07 ± 9.35	0.46
Hemoglobin (g/dL)	15.50 ± 0.62	15.34 ± 1.90	15.36 ± 1.06	0.46
ESR	13.00 ± 5.29	11.28 ± 7.88	11.00 ± 6.60	0.60
AST (IU/L)	27.91 ± 9.83	25.31 ± 8.67	24.65 ± 7.43	0.70
ALT (IU/L)	29.27 ± 18.93	31.70 ± 18.87	30.30 ± 14.93	0.68
rGTP (IU/L)	41.64 ± 39.71	53.42 ± 53.89	46.39 ± 36.35	0.38
FBS (mg/dL)	99.09 ± 12.68	104.88 ± 24.60	105.43 ± 25.93	0.82
Uric acid (mg/dL)	6.11 ± 1.34	6.03 ± 1.49	5.95 ± 1.32	0.95
Triglycerides (mg/dL)	109.09 ± 50.32	179.51 ± 141.44	171.10 ± 110.21	0.09
Total cholesterol (mg/dL)	189.73 ± 31.04	205.79 ± 39.91	200.51 ± 29.52	0.51
HDL cholesterol (mg/dL)	49.27 ± 15.32	44.30 ± 9.45	42.68 ± 9.54	0.51
hs-CRP (mg/dL)	0.30 ± 0.28	0.24 ± 0.40	0.20 ± 0.40	0.30
Lp (a)	7.26 ± 9.95	9.41 ± 9.60	14.36 ± 20.07	0.41
Free T4 (ng/dL)	1.00 ± 0.16	1.04 ± 0.15	1.21 ± 1.12	0.45
TSH (μg/mL)	3.06 ± 3.31	1.60 ± 1.24	1.85 ± 3.27	0.50
Ferritin (ng/mL)	190.86 ± 208.36	164.29 ± 82.94	183.24 ± 145.98	0.84

All values are presented as mean ± standard deviation of the mean. *P values were obtained by Kruskal-Wallis H test.

SBP: systolic blood pressure, DBP: diastolic blood pressure, ESR: erythrocyte sedimentation rate, AST: aspartate transaminase, ALT: alanine aminotransferase, FBS: fasting blood sugar, hs-CRP: high-sensitivity C-reactive protein, Lp (a): lipoprotein a, and TSH: thyroid stimulating hormone.

TABLE 3: Risk factors for atherosclerosis according to female gender.

	CC	CT	TT	P*
Age	50.10 ± 15.44	48.40 ± 11.69	47.84 ± 10.25	0.93
Waist circumference (cm)	85.09 ± 6.74	87.16 ± 9.07	86.00 ± 8.96	0.79
Body mass index (Kg/m ²)	22.64 ± 2.20	23.45 ± 2.94	23.49 ± 3.24	0.83
SBP (mmHg)	118.12 ± 20.49	123.09 ± 15.39	123.05 ± 15.43	0.65
DBP (mmHg)	70.75 ± 11.11	73.35 ± 8.85	72.74 ± 10.00	0.77
Hemoglobin (g/dL)	13.13 ± 0.61	13.33 ± 0.83	13.26 ± 1.49	0.82
ESR	18.00 ± 16.63	16.53 ± 14.37	17.89 ± 13.38	0.96
AST (IU/L)	21.60 ± 7.44	22.95 ± 11.58	20.30 ± 5.42	0.65
ALT (IU/L)	18.30 ± 7.04	21.51 ± 17.93	18.98 ± 8.98	0.96
rGTP (IU/L)	18.90 ± 14.05	19.90 ± 11.49	18.41 ± 14.30	0.59
FBS (mg/dL)	93.10 ± 9.69	96.77 ± 12.65	98.96 ± 23.63	0.85
Uric acid (mg/dL)	4.58 ± 0.73	4.53 ± 1.11	4.17 ± 0.86	0.29
Triglycerides (mg/dL)	111.30 ± 64.14	110.87 ± 54.75	114.57 ± 62.84	0.97
Total cholesterol (mg/dL)	193.50 ± 32.53	200.38 ± 38.01	184.91 ± 36.23	0.18
HDL cholesterol (mg/dL)	57.40 ± 14.51	49.36 ± 9.46	46.11 ± 7.86	0.05*
hs-CRP (mg/dL)	0.15 ± 0.13	0.08 ± 0.05	0.24 ± 0.58	0.35
Lp (a)	18.95 ± 4.56	17.26 ± 18.15	18.33 ± 12.75	0.46
Free T4 (ng/dL)	0.96 ± 0.19	1.00 ± 0.16	1.07 ± 0.17	0.10
TSH (μg/mL)	1.49 ± 2.06	2.56 ± 2.96	1.43 ± 2.37	0.05*
Ferritin (ng/mL)	63.58 ± 44.21	58.77 ± 45.86	80.91 ± 56.66	0.42

All values are presented as mean ± standard deviation of the mean. *P values were obtained by Kruskal-Wallis H test.

SBP: systolic blood pressure, DBP: diastolic blood pressure, ESR: erythrocyte sedimentation rate, AST: aspartate transaminase, ALT: alanine aminotransferase, FBS: fasting blood sugar, hs-CRP: high-sensitivity C-reactive protein, Lp (a): lipoprotein a, and TSH: thyroid stimulating hormone.

TABLE 4: Risk factors for atherosclerosis according to CD 14 -159 genotypes in subjects with *H. pylori* infection.

	CC	CT	TT	P*
Age	43.15 ± 7.16	47.31 ± 10.45	46.24 ± 9.80	0.38
Waist circumference (cm)	89.36 ± 7.67	88.98 ± 8.91	87.05 ± 7.29	0.42
Body mass index (Kg/m ²)	24.85 ± 2.66	24.21 ± 3.09	24.14 ± 2.93	0.73
SBP (mmHg)	127.85 ± 15.15	125.35 ± 13.58	125.31 ± 12.96	0.82
DBP (mmHg)	78.00 ± 11.80	75.27 ± 8.82	75.67 ± 9.52	0.64
Hemoglobin (g/dL)	14.90 ± 1.23	14.91 ± 1.45	14.54 ± 1.65	0.39
ESR	17.71 ± 1.33	14.70 ± 1.44	16.74 ± 1.15	0.83
AST (IU/L)	28.38 ± 9.80	24.19 ± 10.15	23.78 ± 5.68	0.33
ALT (IU/L)	28.23 ± 18.10	26.50 ± 16.39	28.30 ± 13.54	0.21
rGTP (IU/L)	38.85 ± 37.65	49.73 ± 59.32	32.28 ± 24.49	0.31
FBS (mg/dL)	99.23 ± 11.53	103.67 ± 24.49	101.74 ± 22.84	0.78
Uric acid (mg/dL)	5.85 ± 1.30	5.44 ± 1.51	5.47 ± 1.66	0.68
Triglycerides (mg/dL)	105.92 ± 49.02	164.89 ± 108.10	151.50 ± 108.60	0.18
Total cholesterol (mg/dL)	199.92 ± 29.42	201.55 ± 38.49	196.39 ± 29.44	0.72
HDL cholesterol (mg/dL)	50.15 ± 13.47	45.16 ± 8.62	44.98 ± 9.91	0.20
hs-CRP (mg/dL)	0.28 ± 0.25	0.16 ± 0.18	0.27 ± 0.56	0.59
Lp (a)	9.82 ± 11.34	13.19 ± 16.07	12.26 ± 14.86	0.88
Free T4 (ng/dL)	0.96 ± 0.16	1.01 ± 0.14	1.08 ± 0.32	0.16
TSH (μg/mL)	3.34 ± 3.31	1.59 ± 1.32	2.00 ± 3.88	0.13
Ferritin (ng/mL)	168.80 ± 181.46	152.82 ± 96.51	110.18 ± 53.60	0.26

All values are presented as mean ± standard deviation of the mean. *P values were obtained by one-way ANOVA.

SBP: systolic blood pressure, DBP: diastolic blood pressure, ESR: erythrocyte sedimentation rate, AST: aspartate transaminase, ALT: alanine aminotransferase, FBS: fasting blood sugar, hs-CRP: high-sensitivity C-reactive protein, Lp (a): lipoprotein a, and TSH: thyroid stimulating hormone.

cardiovascular diseases is continuously being accumulated [35–37].

Although this study includes small size of the study population, it might be difficult to figure out the association between genetic polymorphism and disease phenotype. Also, we did not consider risk factors such as exercise activity and diet pattern. Especially, these are the important modifiable and preventable risk factors for cardiovascular disease. Our result showed that significant difference of HDL cholesterol level was pronounced in female population, because of the possibility that these contributing factors for atherosclerosis were excluded in female. In our results, there was significant difference in TSH level among the three genotypes. Although the high level of serum TSH level is associated with multivessel disease, it was not the determinant of cardiovascular disease in patients with normal range of thyroid function [38, 39]. Moreover, it cannot exclude a type 2 statistical error. To overcome it, the researcher will sample as many subjects as cost and time allow.

In the last two decades, epidemiologic studies have demonstrated that atherosclerosis is associated with several infectious pathogens, including *H. pylori*, and the existence of a positive association between *H. pylori* and CAD [2–5]. In addition, a modest influence on CAD and progressive atheroma could be caused by *H. pylori* infection [40, 41]. However, it could be debatable due to confounding bias and influenced by the degree of investigations heterogeneity. On the other hand, individual susceptibility for specific disease existed, and genetic polymorphism can be associated with pathogenetic mechanism. Previously, specific genotype of CD14 gene may favor increased inflammation in atheroma, promoting possible worsening atherosclerosis [18–22]. We hypothesized that genetic susceptibility to *H. pylori* infection is associated with the risk of atherosclerosis and aimed to evaluate risk factors for atherosclerosis according to the CD14 polymorphism in healthy subjects with *H. pylori* infection. In our results, the specific genotype of CD14 gene seems to have the risk of atherosclerosis. However, CD14 polymorphism was associated neither with the rate of *H. pylori* infection nor with *H. pylori* induced atherosclerotic risk response in Korean population. There were several limitations in this study. We could not rule out the possibility that some cases were negative for *H. pylori* at the time of collection of tissue sample. Also, we did not consider the specific pathogenic strain because the association of chronic *H. pylori* infection with risk of atherosclerosis appeared to be limited to *cagA* bearing strains in previous study [42, 43]. Its clinical significance is uncertain and these results should expand the sample to do further research. In addition, whether eradication of *H. pylori* might decrease the risk of atherosclerosis should be evaluated.

To date, there are limited data regarding the probability of cardiovascular diseases according to CD14 genetic polymorphisms in Korean population. This provides a clue that the high risk individuals of cardiovascular diseases are discriminated using genetic analysis. In conclusion, the promoter polymorphism at –159C/T of the CD14 gene is

positively associated with the risk factor of atherosclerosis in healthy Korean population. Broader studies will be required before any relatively concrete conclusions can be drawn.

Conflict of Interests

There is no conflict of interests.

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Research Article

The Clinical Correlations of *Helicobacter pylori* Virulence Factors and Chronic Spontaneous Urticaria

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Background and Study Aims. The association between *Helicobacter pylori* (*H. pylori*) and chronic spontaneous urticaria (CSU) remains controversial. This study explored the role of *H. pylori* in CSU among different virulent genotypes patients. **Patients and Methods.** Patients infected by *H. pylori* were sorted into two groups as group A (with CSU) and group B (without CSU). The tissue materials were taken via endoscopy for polymerase chain reaction study to determine virulence factors. After *H. pylori* eradication therapy, the eradication rate and response of urticaria were evaluated by using C¹³-UBT and a three-point scale (complete remission, partial remission, or no improvement). **Results.** The results were comparable between patients of groups A and B in terms of *H. pylori* infection rates and eradication rate. Longitudinal follow-up of 23.5 months showed complete remission of urticaria in 63.6% but no improvement in 36.4% of the patients after *H. pylori* eradication. *H. pylori* infected patients with different virulence factors such as cytotoxin-associated gene A, vacuolating cytotoxin gene A signal region and middle region have similar remission rates for CSU. **Conclusions.** Current study suggests that *H. pylori* may play a role in the development and disease course of CSU but may be irrelevant to different virulent genotypes.

1. Introduction

Chronic spontaneous urticaria (CSU), defined as spontaneous occurrence of wheal and/or angioedema lasting for a period of longer than 6 weeks, is a common and often frustrating problem, affecting up to 1 percent of the general population [1, 2]. The causes of CSU are numerous; however, in at least 80–90% of the patients, the etiology is

undetermined [2, 3]. Recent data show that about 30% of the affected patients may have functional autoantibodies [4]. On the other hand, *Helicobacter pylori* (*H. pylori*) infection is probably the most common chronic bacterial infection in humans, with the prevalence rate in general population estimated to be around 50% in developing countries [5]. It has been generally accepted that *H. pylori* infection plays an etiologic role in the development of chronic active gastritis,

peptic ulcer disease, gastric malignancy, and low-grade gastric mucosa-associated lymphoid tissue lymphoma [5–8]. *H. pylori* is genetically highly diverse, and several genotypes have been identified to associate with severe gastric mucosal inflammation [9]. Cytotoxin-associated gene A (*cagA*) and vacuolating cytotoxin gene A (*vacA*), the two most important virulence factors of *H. pylori* [9], have been reported to enhance its pathogenicity [10] while *cagA* is related to peptic ulcer and gastric malignancy in certain populations [11, 12]; *vacA* can induce host cell vacuolation and eventually cell death [11]. A high degree of sequence variability exists in the *vacA* gene, with its signal and middle region being classified into *s1/s2* and *m1/m2* subtypes, respectively [13]. The *s1* subtype and *m1* subtype have been linked to more severe gastrointestinal manifestations [14].

A potential association between CSU and *H. pylori* infection of the upper gastrointestinal tract has been proposed, but the studies so far showed controversial results [15–18]. Moreover, little is known about the association between the genotypes of *H. pylori* and CSU [15]. This study aimed to explore the potential role of *H. pylori* in the development and disease course of CSU among the different virulent genotypes of patients.

2. Patients and Methods

2.1. Study Design. From August 2008 to July 2009, 25 patients (age 27–68 years, mean = 45.5 years, female/male = 13/12) diagnosed as CSU with unremarkable findings in allergy diagnostics (basis examination) were recruited from the Dermatology Outpatient Department of Chang Gung Memorial Hospital-Kaohsiung, Taiwan [1]. The duration of CSU ranged between 6 and 360 months with a median of 12 months. Only six of them (6/25, 24%) suffered from upper gastrointestinal symptom. All of them received a C^{13} -urea breath test (C^{13} -UBT). Infection of *H. pylori* was diagnosed by a positive C^{13} -UBT test and sorted as group A ($n = 14$). Meanwhile, 24 patients (age 18–83 years, mean = 41.5 years, female/male = 13/11) with gastrointestinal symptoms but without urticaria/pruritus were enrolled from the Gastroenterology Department for C^{13} -UBT examination, and the infected patients were categorized into group B. All the C^{13} -UBT-positive patients underwent upper gastrointestinal endoscopy, using a GIF XQ 240 endoscope (Olympus Optical Company, Tokyo, Japan), and tissue biopsies were taken from the gastric antrum and body ($n = 14$). Criteria for exclusion included (a) ingestion of antibiotics, bismuth, or proton-pump inhibitors within the prior 4 weeks, (b) use of non-steroidal anti-inflammatory drugs within the prior 4 weeks, (c) patients with previous gastric surgery, (d) the coexistence of serious concomitant illness (e.g., decompensated liver cirrhosis or uremia), (e) pregnant women, and (f) those who refused endoscopic examination and subsequent *H. pylori* eradication. *H. pylori* infection was defined as positive results by a positive C^{13} -UBT test.

All the biopsied specimens were stored in 70% ethanol in Eppendorf tubes at -80°C until processed for polymerase chain reaction (PCR) examination, in which the tissue

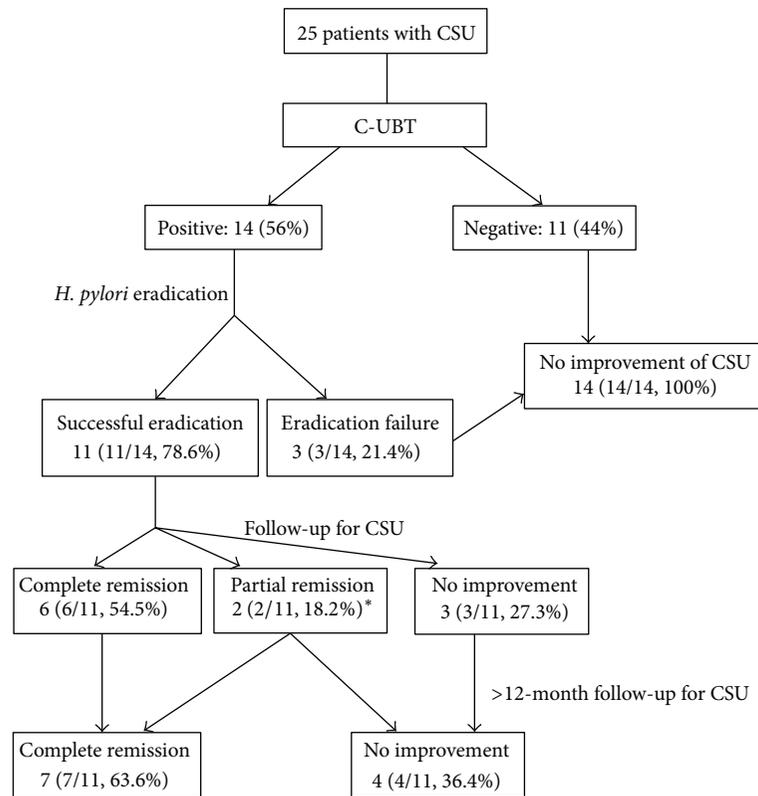
specimens were homogenized with a sterile micropestle, and the DNA was extracted and purified using a commercial kit following the tissue protocol of the manufacturer (QIAGEN QIAamp DNA mini kit, Hilden, Germany). To detect the virulence factors of *H. pylori*, PCR studies with three respective species-specific primer sets were designed to amplify highly conserved regions within the genes encoding *cagA* and *vacA* (*s* and *m* regions) [19].

2.2. Treatment Allocation. All the infected patients then received oral eradication therapy comprising esomeprazole (40 mg twice daily), clarithromycin (500 mg twice daily), and either amoxicillin (1 gm twice daily) or metronidazole (500 mg twice daily) for patients with penicillin allergy in the history [12]. Esomeprazole and amoxicillin were taken one hour before breakfast and dinner, clarithromycin and metronidazole twice daily after breakfast and dinner. To assess eradication efficacy, a repeated C^{13} -UBT was performed to each patient at six weeks after the end of anti-*H. pylori* therapy. The effectiveness of eradication therapy on CSU was assessed three months later after treatment, using a three-point rating scale, that is, complete remission, partial remission (50% or more), or no improvement. The differences in *cagA*, *vacA s*, and *vacA m* of *H. pylori* between patients of group A and group B as well as the differences in clinical course of CSU before and after eradication therapy relating to the various virulent factors were analyzed. The study was approved by the ethic committee of Chang Gung Memorial Hospital-Kaohsiung, Taiwan (no. 95-1314B), and signed informed consent was obtained from all the participants.

2.3. Statistical Analysis. Continuous variables, given as means and standard deviations (SD), were analyzed using the Mann-Whitney *U* test. Categorical variables, given in total and as percentages, were analyzed using the Chi-square test or Fisher exact test. Two-sided *P* values of <0.05 were considered significant. All the statistics were performed using SPSS (WIN version 15.0).

3. Results

Allocation diagrams of patients with chronic spontaneous urticaria are summarized in Figure 1. Demographic data of *H. pylori*-infected patients with or without chronic spontaneous urticaria (CSU) was summarized in Table 1. In group A, clinical follow-up of 11 successfully treated patients three months later revealed complete remission of urticaria in 54.5% (6/11), partial remission in 18.2% (2/11), and no improvement in 27.3% (3/11). In longitudinal follow-up studies for a duration of 12–29 months after *H. pylori* eradication (median = 23.5 months), complete remission was found in 63.6% (7/11) and no improvement in 36.4% (4/11) of the patients. One patient with partial remission turned into complete remission while another one showed deterioration of urticaria. All the three patients in group A who failed *H. pylori* eradication showed clinically persisting urticarial symptoms. The duration of urticaria was 14.9 ± 5.8 months in the treatment responders as compared to 15.1 ± 8.8 months in the nonresponders.



-CSU: chronic spontaneous urticaria; C-UBT: C¹³-urea breath test;
H. pylori: *Helicobacter pylori*

* One patient with partial remission turned into complete remission while another one showed deterioration of urticaria

FIGURE 1: Allocation diagrams of patients with chronic spontaneous urticarial.

TABLE 1: Demographic data of *H. pylori*-infected patients with or without chronic spontaneous urticaria (CSU).

	Group A (with urticaria) N = 14	Group B (without urticaria) N = 14	P value
Age (years)	41.2 ± 11.7	47.8 ± 7.6	0.114
Gender (male/female, %)	50	35.7	0.704
Success rate of <i>H. pylori</i> eradication (%)	11 (78.6)	10 (71.4)	1.0
Genotype			
cagA (%)	11 (78.6)	13 (92.9)	0.596
vacAs1/s2 (%)	11 (78.6)/3 (21.4)	13 (92.9)/1 (7.1)	0.596
vacAml/m2 (%)	6 (42.9)/8 (51.7)	2 (14.3)/12 (85.7)	0.209

However, there was no statistical difference observed in the clinical response rates of CSU between the treatment success and treatment failure patients (7/11, 63.6% versus 0/3, 0%, $P = 0.193$, Fisher’s exact test). None of the patients in group B developed urticarial lesions in an average follow-up of 14.1 months.

Determination of virulence factors via PCR study showed the size of the amplified products as follows: cagA (324 bp), vacA s1 (259 bp), vacA s2 (286 bp), vacA m1 (290 bp), and vacA m2 (352 bp) (Figure 2). The cagA genotype was detected in 11/14 (78.6%) in group A and 13/14 (92.9%) in group B ($P = 0.596$). The ratio of s1 to s2 alleles in vacA was 11 (78.6%) to 3 (21.4%) in group A and 13 (92.9%) to 1 (7.1%) in group B (78.6% versus 92.9%, $P = 0.596$). The ratio of m1 to m2 alleles was 6 (42.9%) to 8 (51.7%) in group A and 2 (14.3%) to 12 (85.7%) in group B (42.9% versus 14.3%, $P = 0.209$). The expression of cagA, vacA (s1/s2), and vacA (m1/m2) did not differ between patients in group A and group B ($P = 0.596$, 0.596, and 0.209, resp.) (Table 2). In analysis of the association between virulence genotypes and therapeutic response of urticaria (group A), the cagA genotype was detected in 5 (71.4%) of 7 CSU patients with remissions (complete and partial) and 6 (85.7%) of 7 patients without improvement ($P = 1.0$). The s1/s2 ratio of vacA genotype was 5 (71.4%) to 2 (28.6%) in remission patients and 6 (85.7%) to 1 (14.3%) in nonremission patients ($P = 1.0$). The ratio of m1 to m2 alleles in vacA was 3 (42.9%) to 4 (57.1%) with remission of CSU posttreatment and 3 (42.9%) to 4 (57.1%) with persisting CSU ($P = 1.0$). As summarized in Table 2, there was no significant difference observed between CSU patients in remission and

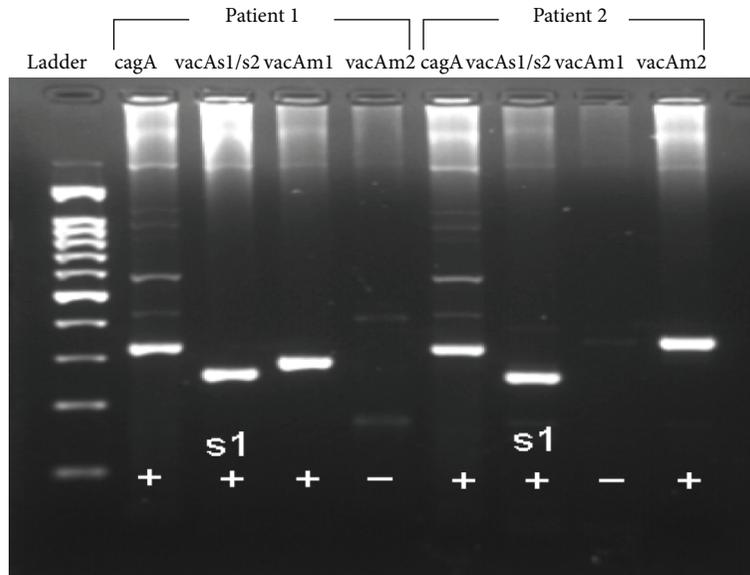


FIGURE 2: Results of PCR study showed the amplification products of *Helicobacter*-specific virulence factors *cagA* (324 bp), *vacA* s1 (259 bp), *vacA* s2 (286 bp), *vacA* m1 (290 bp), and *vacA* m2 (352 bp) such as patient 1 with CSU and positive genotypes *cagA* and *vacA* s1m1 and patient 2 (control group) with positive expression of *cagA* and *vacA* s1m2 but without CSU.

TABLE 2: The expression of virulence factors for CSU patients in remission and nonremission status after successful *H. pylori* eradication treatment.

Genotype	Remission (N = 7)	Nonremission (N = 7)	P value
CagA	5 (71.4)	6 (85.7)	1.0
VacAs1/s2	5 (71.4)/2 (28.6)	6 (85.7)/1 (14.3)	1.0
VacAm1/m2	3 (42.9)/4 (57.1)	3 (42.9)/4 (57.1)	1.0
VacA s1m1	2 (28.6)	3 (42.9)	0.5
CagA + VacA s1m1	2 (28.6)	3 (42.9)	0.5

nonremission status after successful *H. pylori* eradication treatment for the expression of virulence factors such as *cagA*, *vacA* (s1/s2), *vacA* (m1/m2), *vacA* s1m1, or (*cagA* + *vacA*) s1m1.

4. Discussion

A potential association between CSU and *H. pylori* infection of the upper gastrointestinal tract has been proposed, but the studies so far showed controversial results not to mention the relevance to the genotypes of *H. pylori* [15–18]. In current study, the prevalence rate of *H. pylori* infection of 56% in our CSU patients which is rational as reported in other studies varied from 47% to 80% [20–23]. All of these studies, including ours, also observed that the prevalence of *H. pylori* infection between patients with and without CSU is similar. Moreover, follow-up of the 14 infected patients with gastric complaints but without skin problems (group B) for an average of 14.1 months did not find a later development of CSU. However, clinical regression of CSU was observed after

a successful eradication of *H. pylori*, among 17.6% to 88% of these patients [20–23]. 58.3% of our CSU patients had a long-term complete remission of their CSU after an effective eradication therapy. Given that a relatively low reported spontaneous remission rate of 6% in the natural course of CSU persisting for more than 6 months [24], current study demonstrated an apparent benefit from *H. pylori* eradication therapy for CSU patients and suggested that a possible etiopathogenetic role of *H. pylori* may exist.

H. pylori species is genetically highly diverse [9]. Differences in the genotypes of bacterial virulence factors can induce variable degrees of gastric inflammation and elicit different clinical manifestations in gastrointestinal tract. The strains have been categorized into type I with expression and type II without expression of the virulence factors [25]. Type I *H. pylori* can cause mucosal damage by stimulating gastric epithelial cytokine responses and produce a variety of other factors that determine the local inflammatory response [26]. Among the well-characterized virulence factors of type I strains, *cagA*, *vacA*-s, and *vacA*-m have been reported to enhance pathogenicity of *H. pylori* [10]. CagA-positive strains induce a higher production of proinflammatory cytokines in the gastric mucosa and are linked with an increased risk of peptic ulcer disease, gastric atrophy, and gastric cancer in certain populations [27, 28]. VacA, which is produced by 50–60% of *H. pylori* strains, causes fusion of the endocellular lysosomes leading to a consequent reswelling of the gastric epithelium cells [27]. *H. pylori* bacteria carrying *vacA*-s1 and *vacA*-m1 subtypes have also been related to more severe clinical manifestations [9, 27].

There is proposal of potential association between CSU and *H. pylori* infection of the upper gastrointestinal tract [15–18], but only one study reported the clinical relevance between virulence factors of *H. pylori* and CSU [15]. Fukuda

and his colleagues found a high incidence of *cagA* expression in CSU patients (100%, 13/13) and similarly in control group (100%, 26/26) [15]. This was the same to current study. Longitudinal follow-up (range 12–29 months, median 23.5 months) showed complete remission of urticaria in 63.6% (7/11) and no improvement in 36.4% (4/11) of our patients after *H. pylori* eradication. In addition, we had shown that the expression of other virulence factors did not differ between patients with and without CSU. *H. pylori* infected patients with different virulence factors such as cytotoxin-associated gene A, vacuolating cytotoxin gene A signal region and middle region had similar remission rates for CSU. Moreover, among CSU patients, the genotypes of *H. pylori* virulence factors did not correlate with the onset age, gender difference, or the response to bacterial eradication therapy.

H. pylori colonizes the gastric mucosa in approximately half of the world's population, but only a minority (10–20%) of the infected individuals develop clinical manifestations, most commonly with gastrointestinal disorders [7, 27]. There were debates on the clinical association of *H. pylori* with certain dermatological disorders which includes issues such as CSU, rosacea, psoriasis, or immune thrombocytopenic purpura [25, 29]. Like peptic ulcer disease, rosacea may be triggered by the more virulent type I strain of *H. pylori* [30]. It is also believed that many environmental, bacterial, and host-related factors can influence the course of infection. The *cagA* protein of the more virulent strain stimulates the gastric epithelium to secrete greater amounts of inflammatory cytokines such as IL-8, IL-1, TNF- α , interferon- γ , leukotrienes, and platelet-activating factors [30]. On the other hand, it is suggested that *H. pylori* infection may facilitate penetration of allergens with induction of an IgE response to certain common alimentary antigens thereby enhancing the development of food allergy [31]. Infection with *H. pylori* has been shown to increase antigen absorption across the cultured digestive epithelium *in vitro* and across the gastric mucosa of mice *in vivo* [32, 33]. Nevertheless, it is still unclear whether the immunologic reactions induced by *H. pylori* can directly elicitate the mast cell degranulation leading to urticarial formation.

Current study encounters some limitations. First, the sample size is relatively small so bias may still exist. Second, there is no matched control group for this study which should be untreated from *H. pylori* infections. The bottom line is that it is practically unethical not to treat a diagnosed infection. Third, there are other virulence factors of *H. pylori* which were not studied, such as *iceA*, *babA*, *flaA/flaB* (the genes for flagellins), and *ureA/ureB* (urease-encoding genes). As we know, *iceA* is induced by contact with epithelium; *babA* is associated with binding to blood-group antigens [34, 35]. However, unlike *cagA*, *vacA-s1*, and *-m1*, *iceA1* and *babA2* which are associated with a more severe gastrointestinal manifestation [35, 36], *flaA/flaB* and *ureA/ureB* genotypes are of less clinical significance [37].

5. Conclusions

Current study suggests that *H. pylori* may play a role in the development and disease course of CSU. Different virulent

genotypes of *H. pylori* may be irrelevant to the remission of CSU after eradication. However, it remains to be determined whether a quantitative effect of the examined virulence factors may exist or other virulent and nonvirulent genes of *H. pylori* may play a role in the pathogenesis of CSU. A better understanding of the bacterial virulence factors and the corresponding host immune response is still needed to further clarify the pathogenic role of *H. pylori* in certain groups of patients with CSU.

Conflict of Interests

The authors declare that they have no conflict of interests.

Acknowledgments

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