Journal of Infection and Public Health (2012) 5, 153—158

http://www.elsevier.com/locate/jiph

Helicobacter pylori genotypes can predict gastric tissue histopathology: A longitudinal study of Iranian patients

Hossein Khedmat[a,b,*], Ali Karami[b,c], Zahra Safiri[b,c], Mohsen Amini[a,b], Ali Bakhtiari[b], Ashraf Karbasi[a,b], Mojgan Jayhounian[b,d], Hamidreza Jalalian[e], Saeed Taheri[f]

[a] *Baqiyatallah Research Center for Gastroenterology & Liver Diseases, Tehran, Iran*
[b] *Baqiyatallah University of Medical Sciences, Tehran, Iran*
[c] *Biology Research Center, Tehran, Iran*
[d] *Department of Pathology, Baqiyatallah University of Medical Sciences, Tehran, Iran*
[e] *Department of Medicine, Baqiyatallah University of Medical Sciences, Tehran, Iran*
[f] *Dr Taheri Medical Research Group, Tehran, Iran*

Received 5 January 2011; received in revised form 22 August 2011; accepted 10 October 2011

KEYWORDS
PCR;
Helicobacter pylori;
Gastritis;
Diagnosis;
Vulnerability

Summary
Introduction: Several factors have been suggested to account for differences in the virulence of *Helicobacter pylori* infections in various populations. Evidence suggests the existence of different strains of *H. pylori* with different degrees of virulence. The present study aimed to investigate the gastric histopathology in Iranian patients infected with *H. pylori* and to investigate the relationship between the severity of gastritis and four different bacterial virulence-associated genotypes.
Methods and materials: All of the patients with positive results from a pathological examination, a rapid urease test, and PCR analysis for *H. pylori* infection were consecutively included into the study. The classification and grading of gastritis were performed according to the Sydney System. Esophagitis was classified endoscopically according to the Savary—Miller grading system. The primers used in this study targeted 16S rRNa (521 bp), Urease A (411 bp), Cag A (400 bp), and 26 kDa (303 bp).

Abbreviations: H. pylori, *Helicobacter pylori*; PCR, polymerase chain reaction.

* Corresponding author at: Baqiyatallah Research Center for Gastroenterology & Liver Diseases, Baqiyatallah Hosp., Mullasadra St, Tehran, P.O. Box 14155-6437, postal code: 1435915371, Iran.
E-mail address: Khedmat.h@gmail.com (H. Khedmat).

1876-0341/$ — see front matter. Crown Copyright © 2012 Published by Elsevier Limited on behalf of King Saud Bin Abdulaziz University for Health Sciences. All rights reserved.
doi:10.1016/j.jiph.2011.10.009

Results: Twenty-eight patients were included in the study. The presence of Cag A showed a significant relationship with higher gastritis grades (3.0 ± 0.7 vs. 2.3 ± 0.9, $p = 0.024$) and higher scores for *H. pylori* infection (3.0 ± 0.7 vs. 2.3 ± 0.7, $p = 0.027$). The patients infected with 26 kDa-positive *H. pylori* had significantly higher infection scores (3.5 ± 0.6 vs. 2.5 ± 0.6, $p = 0.020$).
Conclusion: This study showed that CagA-positive *H. pylori* infection is associated with more severe gastritis and with increased bacterial density and inflammation in the biopsy specimens. The 303-bp positive genotype was also significantly associated with higher grades of esophagitis. Additional in-depth trials will be helpful in extending our findings.
Crown Copyright © 2012 Published by Elsevier Limited on behalf of King Saud Bin Abdulaziz University for Health Sciences. All rights reserved.

Introduction

Helicobacter pylori is a Gram-negative spiral flagellate bacillus that lives in the gastric mucus that is adherent to the mucosa. *H. pylori* infects almost half of the world's population, producing a chronic infection that can lead to gastric and duodenal ulcers, gastric cancer, and B-cell mucosa-associated lymphoid tissue lymphoma [1—3]. This pathogen colonizes the human stomach and establishes a long-term infection of the gastric mucosa [4].

Current knowledge indicates that the prevalence of *H. pylori* colonization and the prevalence of related diseases are not directly correlated. Several factors have been suggested to account for this observation, including genetic diversity among individuals [5], environmental factors that include age at the time of first infection, nutrition, and the genetic variability of *H. pylori* [6,7]. Despite the highly heterogenic nature of *H. pylori* genetics [8], distinct genetic lineages have been shown to differ with respect to pathogenicity [9].

Evidence suggests that there exist different strains of *H. pylori* with different degrees of virulence [10—13]. The cytotoxin-associated gene (cagA) is a marker for a genomic pathogenicity island of c. 40 kb (cag-PAI) and is reported to be associated with increased interleukin 8 production and mucosal inflammation; therefore, cagA is associated with more severe clinical outcomes [14]. Several other genes have also been studied and found to be correlated with the histopathological findings for *H. pylori*-induced gastritis [15,16,14]. On the other hand, some studies have also detected no relationship or even the converse findings [17,18].

The present study aimed to investigate the gastric histopathology of Iranian patients infected with *H. pylori* and to investigate the histopathological relationship with four different bacterial virulence associated genotypes.

Methods and material

Patients

The patients who were admitted to the gastroenterology outpatient clinic with dyspepsia and underwent a diagnostic endoscopic evaluation with biopsy from March 2005 to March 2006 were examined to determine if they were infected with *H. pylori*. Those subjects who had concomitant positive results for the pathological examination, the rapid urease test, and the PCR analysis were consecutively included into the study. Biopsies were taken from the antrum of the patients for the rapid urease test, pathological examination, and DNA analysis. The first specimen was rapidly sent to the pathology department for grading and evaluation, two other specimens were sent to the pathology clinic, and the remaining specimen was frozen for PCR analysis.

All of the patients with positive results for the pathological examination, the rapid urease test, and the nested PCR methods designed to detect *H. pylori* infection were included in the analysis. The patient samples were considered positive for *H. pylori* if one of the 521 bp, 411 bp, 400 bp, or 303 bp bands was observed in the PCR reaction. Ten samples (36%) showed the 400 bp band, 8 (29%) contained the 303 bp band, 12 (43%) contained the 521 bp band, and 13 (46%) exhibited the 411 bp band.

Histological and clinical grading of gastritis and esophagitis

Paraffin-embedded tissue sections were stained with hematoxylin and eosin to grade the severity of gastritis and with Giemsa stain to detect *H. pylori*. The classification and grading of gastritis were performed according to the Sydney System [19] by an experienced pathologist who did not know the bacterial genotype.

The degrees of acute and chronic inflammation and the microbial density were scored from 0 to 5 and used as the ''*H. pylori* infection score''. Esophagitis was classified endoscopically according to the Savary—Miller grading system [20].

Rapid urease test

One antrum biopsy specimen was introduced into semisolid 2% urea agar with a sterile needle and incubated at room temperature. The results were recorded up to 4 h after inoculation [21].

Preparation of samples for PCR amplification

Genomic DNA was extracted from all of the strains with the method described in Marais et al. [22]. The extracted DNA was dissolved in water, and stock solutions were prepared and used throughout the study. Briefly, the biopsy samples were ground and centrifuged for 5 min at 10 000 $\times$ *g*. After the supernatants were discarded, the biopsy specimens were resuspended in 300 μL of extraction buffer (20 mmol/L Tris—HCl, pH 8.0, 0.5% Tween 20), and proteinase K (0.5 mg/mL final concentration). The mixture was incubated at 56 °C for 1 h, after which the enzyme was inactivated by boiling for 10 min.

We were able to detect *H. pylori*-specific sequences at an estimated concentration of 20 pmol using our nested assay. Five microliters of DNA was used as the template for each PCR. Each sample was amplified by four different PCRs. The primers used in this study were specific for 16S rRNA (521 bp), Urease A (411 bp), Cag A (400 bp), and 26 kDa (303 bp). The primer sequences, conditions, and sizes of the PCR products are listed in Table 1.

Statistical analysis

SPSS software (Statistical Product and Services Solutions, version 13.0, SPSS Inc, Chicago, IL, USA) was used to analyze the data. The statistical significance of the differences between the patient subgroups was assessed using the chi-square test, the Fisher's exact test for proportions, and the *t*-test for continuous data. The Kolmogorov—Smirnov test was used to evaluate the distribution of the data, which was normal for all of the variables assessed. Nonetheless, we reanalyzed the data with non-parametric tests, which did not change the primary results. *p*-Values that were less than .05 were considered statistically significant.

Results

Twenty-eight patients (14 males and 14 females) were included in the study. The mean age of the patients was 48.8 $\pm$ 14.4 years, and the mean body mass index (BMI) was 25.2 $\pm$ 6.2 kg/m^2.

The age, gender, and BMI of the patients were equivalent in all of the *H. pylori*-infected groups (Table 2). We analyzed the potential relationship between the grades of gastritis and duodenitis and the four *H. pylori* genes investigated in the PCR. Student's *t*-test showed no significant relationship between the grade of gastritis and positive PCR results for the 16S rRNa ($p = 0.273$), Urease A ($p = 0.665$), or 26 kDa ($p = 0.286$) genes. The presence of Cag A showed a significant relationship with higher gastritis grades (3.0 $\pm$ 0.7 vs. 2.3 $\pm$ 0.9, $p = 0.024$, standard error: 0.325).

We also analyzed the relationship between the *H. pylori* infection score and the different *H. pylori* genotypes. The patients infected with 16S rRNa-positive ($p = 0.334$), Urease A-positive ($p = 0.541$), or 26 kDa-positive ($p = 0.950$) *H. pylori* did not have different scores than those patients who were infected with *H. pylori* strains without these gene products. Those patients with a Cag A-positive *H. pylori* infection were significantly more likely to exhibit higher *H. pylori* infection scores (3.0 $\pm$ 0.5 vs. 2.3 $\pm$ 0.7, $p = 0.027$, standard error: 0.282).

Finally, we investigated the correlation between esophagitis score and the different *H. pylori* genotypes. The patients infected with 26 kDa-positive *H. pylori* had significantly higher esophagitis scores (3.5 $\pm$ 0.6 vs. 2.5 $\pm$ 0.6, $p = 0.020$, standard error: 0.359). Those patients infected with 16S rRNa-positive ($p = 0.445$), Urease A-positive ($p = 0.108$), or CagA-positive ($p = 0.663$) genotypes did not have esophagitis scores different from the scores of patients with negative genotypes. Barrett's esophagus was not reported in any of the study patients.

Discussion

Different *H. pylori* strains have been shown to predict histopathologically related lesions in gastric mucosa biopsy specimens [23—27]. In our study, the presence of four *H. pylori* strains was investigated in patients with functional dyspepsia, and these results were compared with histological findings.

Although the association between *H. pylori* infection and chronic gastritis is overwhelmingly strong, the development of severe gastric disease is rare in infected individuals. On the other hand, there are some studies that have cast doubt on

Table 1 Primer sequences and expected lengths of the amplified DNA products.

Primer	Length	Sequence
16S rRNa	521 bp	F:5′-GCAATCAGCGTCAGTAATGTTC-3′ R:5′-GCTAAGAGATCAGCCTATGTCC-3′
UreA	411 bp	F:5′-GCCAATGGTAAATTAGTT-3′ R:5′-CTCCTTAATTGTTTTTAC-3′
Cag A	400 bp	F:5′-AATACACCAACGCCTCCAAG-3′ R:5′-TTGTTGCCGCTTTTGCTCTC-3′
26 kDa	303 bp	F:5′-TGGCGTGTCTATTGACAGCGAGC-3′ R:5′-CCTGCTGGGCATACTTCACCATG-3′

the impact of *H. pylori* infection on the severity of gastritis [28]. These variations in the reported clinical consequences of *H. pylori* infection in different groups can be explained by factors such as the duration of the infection, the inflammatory response of the patient, and the virulence of the *H. pylori* strain; these factors differ among different patient groups, leading to inconsistent results for different populations. For example, infection with less virulent strains is associated with mild symptoms and less histopathological damage, whereas infection with more virulent strains is considered to be associated with more severe gastric inflammation and, eventually, peptic ulcers, gastric adenoma, and MALT lymphoma.

The relationship between the *H. pylori* genotype and the gastric inflammatory response to infection varies within nationalities as well. It has been demonstrated that individuals from Western counties develop more severe gastric inflammation after infection with cagA-positive strains [29]. Studies have also shown that the grade of gastritis and the polymorphonuclear density are the highest in individuals infected with cagA-positive strains but are the lowest when cagA-negative organisms colonize the gastric mucosa [23]. Demirturk et al. [26] suggested that cagA positivity is associated with more severe glandular atrophy, inflammation, and activity, whereas Saruc et al. [27] demonstrated a relationship between cagA positivity and inflammation, *H. pylori* density, and intestinal metaplasia, but not between cagA positivity and glandular atrophy. Studies investigating cagA positivity and the histopathological findings of gastritis in Asian countries have produced conflicting results [24,25,30], although the same results have also been reported for studies performed in Western countries [31]. An international study conducted in four Asian and American countries (Japan, Korea, Colombia, and the United States) found no correlation between the presence of cagA, vacA, and iceA, and clinical consequences [32]. In our study, the cagA-positive patients showed more severe gastritis and more severe *H. pylori* infection (characterized by the bacterial density and inflammation). These results are is consistent with the general concept that infection with a cagA-positive *H. pylori* strain impacts the severity of gastritis.

There are scarce published data regarding the impact of infection with 411-bp- and 303-bp-positive strains of *H. pylori*. One explanation for the shortage of data on these strains may be the fact

Table 2 Characteristics of the study patients, grouped according to *H. pylori* genotypes.

Variable	*H. pylori* genotypes				
	16S rRNa	UreA	Cag A	26 kDa	Sig.
Age (y)[b]	45.9 ± 18.2	51.1 ± 19	50.1 ± 13.6	48.6 ± 13.8	NS
Gender (male)[a]	3 (42.8)	3 (50)	4 (57.2)	4 (50)	NS
Body mass index (BMI)[b]	24.5 ± 6.1	24.8 ± 6.8	27.2 ± 6	25 ± 7.1	NS
Infection score[b]	2.3 ± 0.7	2.2 ± 0.9	3.0 ± 0.5	2.4 ± 0.5	0.027[c]
Gastritis grade[c]	2.1 ± 1.1	2.5 ± 0.9	3.0 ± 0.7	2.2 ± 0.4	0.024[c]
Esophagitis[b]	2.3 ± 0.9	2.4 ± 0.5	2.8 ± 0.6	3.5 ± 0.6	0.020[d]

[a] Number (%).
[b] Mean ± SD.
[c] 400 bp vs. other genotypes.
[d] 303 bp vs. other genotypes.

that investigators have failed to find any relationship between the histopathological gastritis findings and these *H. pylori* genotypes, which can make the publication of results more difficult. We also found that the 411-bp genotype had no impact on any of the study variables; however, the patients infected with 303-bp (26 kDa)-positive *H. pylori* strains were significantly more likely to develop more severe esophagitis. Esophagitis has been proposed to be induced by less virulent *H. pylori* strains that possess neither cagA nor iceA, suggesting that virulent strains protect against the development of gastroesophageal reflux and esophagitis [33]. We also found similar results in the current study, with a significantly lower proportion of cagA-positive *H. pylori*-infected individuals developing esophagitis. To the best of our knowledge, 26 kDa has never been reported to be a sensitizing factor for the development of esophagitis, and this report provides the first data illustrating this effect.

In conclusion, this study showed that CagA-positive *H. pylori* infection is associated with more severe gastritis and increased bacterial density, and inflammation in the biopsy specimens. On the other hand, the 303-bp-positive genotype was significantly associated with higher grades of esophagitis. Because of the lack of any co-factors exhibiting a significant association, we were unable to use multivariate analyses. More in-depth trials will be helpful in extending our findings.

Conflict of interest

Authors declare that there is no conflict of interest on the abovementioned article

Funding: All financial support was funded by Baqiyatallah University.

Competing interests: None declared

Ethical approval: Not required

References

[1] Khedmat H, Ahmadzad-Asl M, Amini M, Lessan-Pezeshki M, Einollahi B, Pourfarziani V, et al. Gastro-duodenal lesions and *Helicobacter pylori* infection in uremic patients and renal transplant recipients. Transplant Proc 2007;39(May (4)):1003—7.

[2] Uemura N, Okamoto S, Yamamoto S, Matsumura N, Yamaguchi S, Yamakido M, et al. *Helicobacter pylori* infection and the development of gastric cancer. N Engl J Med 2001;345(11):784—9.

[3] Wotherspoon AC, Doglioni C, de Boni M, Spencer J, Isaacson PG. Antibiotic treatment for low-grade gastric MALT lymphoma. Lancet 1994;343(8911):1503.

[4] Blaser MJ. Ecology of *Helicobacter pylori* in the human stomach. J Clin Invest 1997;100:759—62.

[5] Azuma T, Ito S, Sato F, Yamazaki Y, Miyaji H, Ito Y, et al. The role of the HLA-DQA1 gene in resistance to atrophic gastritis and gastric adenocarcinoma induced by *Helicobacter pylori* infection. Cancer 1998;82:1013—8.

[6] Correa P. Human gastric carcinogenesis: a multistep and multifactorial process—first American Cancer Society Award Lecture on cancer epidemiology and prevention. Cancer Res 1992;52:6735—40.

[7] Varis K, Taylor PR, Sipponen P, Samloff IM, Heinonen OP, Albanes D, et al. Gastric cancer and premalignant lesions in atrophic gastritis: a controlled trial on the effect of supplementation with alpha-tocopherol and beta-carotene. The Helsinki Gastritis Study Group. Scand J Gastroenterol 1998;33:294—300.

[8] Go MF, Kapur V, Graham DY, Musser JM. Population genetic analysis of *Helicobacter pylori* by multilocus enzyme electrophoresis: extensive allelic diversity and recombinational population structure. J Bacteriol 1996;178:3934—8.

[9] Achtman M, Azuma T, Berg DE, Ito Y, Morelli G, Pan ZJ, et al. Recombination and clonal groupings within *Helicobacter pylori* from different geographic regions. Mol Microbiol 1999;32:459—70.

[10] Atherton JC, Cao P, Peek Jr RM, Tummuru MK, Blaser MJ, Cover TL. Mosaicism in vacuolating cytotoxin alleles of *Helicobacter pylori*. Association of specific vacA types with cytotoxin production and peptic ulceration. J Biol Chem 1995;270:17771—7.

[11] Blaser MJ, Perez-Perez GI, Kleanthous H, Cover TL, Peek RM, Chyou PH, et al. Infection with *Helicobacter pylori* strains possessing cagA is associated with an increased risk of developing adenocarcinoma of the stomach. Cancer Res 1995;55(10):2111—5.

[12] Queiroz DM, Mendes EN, Carvalho AS, Rocha GA, Oliveira AM, Soares TF, et al. Factors associated with *Helicobacter pylori* infection by a cagA-positive strain in children. J Infect Dis 2000;181:626—30.

[13] Blaser MJ, Perez-Perez GI, Kleanthous H, Cover TL, Peek RM, Chyou PH, et al. Infection with *Helicobacter pylori* strains possessing cagA is associated with an increased risk of developing adenocarcinoma of the stomach. Cancer Res 1995;55:2111—5.

[14] Audibert C, Burucoa C, Janvier B, Fauchere JL. Implication of the structure of the *Helicobacter pylori* cag pathogenicity island in induction of interleukin-8 secretion. Infect Immun 2001;69:1625—9.

[15] Ikenoue T, Maeda S, Ogura K, Akanuma M, Mitsuno Y, Imai Y, et al. Determination of Helicobacter pylori virulence by simple gene analysis of the cag pathogenicity island. Clin Diagn Lab Immunol 2001;8(1):181—6.

[16] Maeda S, Yoshida H, Ikenoue T, Ogura K, Kanai F, Kato N, et al. Structure of cag pathogenicity island in Japanese *Helicobacter pylori* isolates. Gut 1999;44(3):336—41.

[17] Yamaoka Y, Kodama T, Gutierrez O, Kim JG, Kashima K, Graham DY. Relationship between *Helicobacter pylori* iceA, cagA, and vacA status and clinical outcome: studies in four different countries. J Clin Microbiol 1999;37:2274—9.

[18] Mukhopadhyay AK, Kersulyte D, Jeong JY, Datta S, Ito Y, Chowdhury A, et al. Distinctiveness of genotypes of *Helicobacter pylori* in Calcutta, India. J Bacteriol 2000;182:3219—27.

[19] Price AB. The Sydney system: histological division. J Gastroenterol Hepatol 1991;6:209—22.

[20] Ollyo JB, Lang F, Fontolliet C, Monnier P. Savary—Miller's new endoscopic grading of reflux-oesophagitis: a simple, reproductible, logical, complete and useful classification. Gastroenterology 1990;98(Suppl). p. A100—A100 [abstract].

[21] Deltenre M, Glupczynski Y, De Prez C, Nyst JF, Burette A, Labbé M, et al. The reliability of urease test, histology and culture in the diagnosis of *Campylobacter pylori* infection. Scand J Gastroenterol Suppl 1989;160:19—24.
[22] Marais A, Monteiro L, Occhialini M, Pina M, Lamoliatte H, Megraud F. Direct detection of *Helicobacter pylori* resistance to macrolides by a polymerase chain reaction/DNA enzyme immunoassay in gastric biopsy specimens. Gut 1999;44:463—7.
[23] Nogueira C, Figueiredo C, Carneiro F, Gomes AT, Barreira R, Figueira P, et al. *Helicobacter pylori* genotypes may determine gastric histopathology. Am J Pathol 2001;158: 647—54.
[24] Umit H, Tezel A, Bukavaz S, Unsal G, Otkun M, Soylu AR, et al. The relationship between virulence factors of *Helicobacter pylori* and severity of gastritis in infected patients. Dig Dis Sci 2009;54:103—10.
[25] Li CQ, Pignatelli B, Ohshima H. Coexpression of interleukin-8 and inducible nitric oxide synthase in gastric mucosa infected with cagA1 *Helicobacter pylori*. Dig Dis Sci 2000;45(1):55—62.
[26] Demirturk L, Ozel AM, Yazgan Y, Solmazgul E, Yildirim S, Gultepe M, et al. CagA status in dyspeptic patients with and without peptic ulcer disease in Turkey: association with histopathologic findings. Helicobacter 2001;6:163—8.
[27] Saruc M, Demir MA, Kucukmetin N, Kandiloglu AR, Akarca US, Yuceyar H. Histological and clinical predictive value of determination of tissue CagA status by PCR in *Helicobacter pylori* infected patients; results of the large population based study in western Turkey. Hepatogastroenterology 2002;49:878—81.
[28] Gold BD, van Doorn LJ, Guarner J, Owens M, Pierce-Smith D, Song Q, et al. Genotypic, clinical, and demographic characteristics of children infected with *Helicobacter pylori*. J Clin Microbiol 2001;39(April (4)):1348—52.
[29] Warburton VJ, Everett S, Mapstone NP, Axon AT, Hawkey P, Dixon MF. Clinical and histological associations of cagA and vacA genotypes in *Helicobacter pylori* gastritis. J Clin Pathol 1998;51(January (1)):55—61.
[30] Ho YW, Ho KY, Ascencio F, Ho B. Neither gastric topological distribution nor principle virulence genes of *Helicobacter pylori* contributes to clinical outcomes. World J Gastroenterol 2004;10(November (22)):3274—7.
[31] Husson MO, Gottrand F, Vachee A, Dhaenens L, de la Salle EM, Turck D, et al. Importance in diagnosis of gastritis of detection by PCR of the cagA gene in *Helicobacter pylori* strains isolated from children. J Clin Microbiol 1995;33(December (12)):3300—3.
[32] Yamaoka Y, Kodama T, Gutierrez O, Kim JG, Kashima K, Graham DY. Relationship between *Helicobacter pylori* iceA, cagA, and vacA status and clinical outcome: studies in four different countries. J Clin Microbiol 1999;37(July (7)):2274—9.
[33] Vicari JJ, Peek RM, Falk GW, Goldblum JR, Easley KA, Schnell J, et al. The seroprevalence of cagA-positive *Helicobacter pylori* strains in the spectrum of gastroesophageal reflux disease. Gastroenterology 1998;115(July (1)):50—7; Lai CH, Poon SK, Chen YC, Chang CS, Wang WC. Lower prevalence of *Helicobacter pylori* infection with vacAs1a, cagA-positive, and babA2-positive genotype in erosive reflux esophagitis disease. Helicobacter 2005;10(December (6)):577—85.

Available online at www.sciencedirect.com
SciVerse ScienceDirect