

Diversity of *Helicobacter pylori* genotypes in Iranian patients with different gastroduodenal disorders

Farzam Vaziri, Shahin Najari Peerayeh, Masoud Alebouyeh, Tabassom Mirzaei, Yoshio Yamaoka, Mahsa Molaei, Nader Maghsoudi, Mohammad Reza Zali

Farzam Vaziri, Shahin Najari Peerayeh, Department of Bacteriology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran 14115-331, Iran

Masoud Alebouyeh, Tabassom Mirzaei, Mohammad Reza Zali, Gastroenterology and Liver Diseases Research Center, Shahid Beheshti University of Medical Sciences, Tehran 19835-187, Iran

Yoshio Yamaoka, Department of Environmental and Preventive Medicine, Oita University Faculty of Medicine, Oita 879-5593, Japan

Yoshio Yamaoka, Department of Medicine-Gastroenterology, Baylor College of Medicine, Houston, TX 77030, United States

Mahsa Molaei, Basic and Molecular Epidemiology of Gastrointestinal Disorders Research Center, Shahid Beheshti University of Medical Sciences, Tehran 19835-187, Iran

Nader Maghsoudi, Neuroscience Research Center (NRC), Shahid Beheshti University of Medical Sciences, Tehran 19615-1178, Iran

Author contributions: Najari Peerayeh S, Alebouyeh M and Yamaoka Y contributed equally to this work; Vaziri F performed the research and wrote the paper; Alebouyeh M and Vaziri F designed the research and analyzed the data; Mirzaei T collected the biopsy samples and cultured the bacteria; Molaei M helped this project as a pathologist; Alebouyeh M, Najari Peerayeh S, Maghsoudi N, Yamaoka Y and Zali MR supervised the research; Yamaoka Y edited the manuscript.

Supported by Gastroenterology and Liver Diseases Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran; Iran National Science Foundation, INSF; and a PhD grant from the Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran; Grants-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology (MEXT) of Japan, No. 22390085, 22659087, 24406015 and 24659200; Special Coordination Funds for Promoting Science and Technology from the MEXT of Japan, and a Research Fund at the Discretion of the President, Oita University

Correspondence to: Shahin Najari Peerayeh, PhD, Associate Professor, Department of Bacteriology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran 14115-331, Iran. najarp_s@modares.ac.ir

Telephone: +98-21-82883870 Fax: +98-21-82884555

Received: August 19, 2012 Revised: September 11, 2012

Accepted: November 14, 2012

Published online: September 14, 2013

Abstract

AIM: To investigate the diversity of *Helicobacter pylori* (*H. pylori*) genotypes and correlations with disease outcomes in an Iranian population with different gastroduodenal disorders.

METHODS: Isolates of *H. pylori* from patients with different gastroduodenal disorders were analyzed after culture and identification by phenotypic and genotypic methods. Genomic DNA was extracted with the QIAamp DNA mini kit (Qiagen, Germany). After DNA extraction, genotyping was done for *cagA*, *vacA* (s and m regions), *iceA* (*iceA*₁, *iceA*₂) and *babA* with specific primers for each allele using polymerase chain reaction (PCR). All patients' pathologic and clinical data and their relation with known genotypes were analyzed by using SPSS version 19.0 software. χ^2 test and Fisher's exact test were used to assess relationships between categorical variables. The level of statistical significance was set at $P < 0.05$.

RESULTS: A total of 71 isolates from 177 patients with different gastroduodenal disorders were obtained. Based on analysis of the *cagA* gene (positive or negative), *vacA* s-region (*s*₁ or *s*₂), *vacA* m-region (*m*₁ or *m*₂), *iceA* allelic type (*iceA*₁ and *iceA*₂) and *babA* gene (positive or negative), twenty different genotypic combinations were recognized. The prevalence of *cagA*, *vacA* *s*₁, *vacA* *s*₂, *vacA* *m*₁, *vacA* *m*₂, *iceA*₁, *iceA*₂, *iceA*₁+*iceA*₂ and *babA* were 62%, 78.9%, 19.7%, 21.1%, 78.9%, 15.5%, 22.5%, 40.8% and 95.8%, respectively. Interestingly, evaluation of PCR results for *cagA* in 6 patients showed simultaneous existence of *cagA* variants according to their size diversities that proposed mixed infection in these patients. The most prevalent genotype in *cagA*-positive isolates was *cagA*⁺/*vacA*_{s1m2}/*iceA*₁₊₂/*babA*⁺ and in *cagA*-negative isolates was *cagA*⁻/*vacA*_{s1m2}/*iceA*⁻/*babA*⁺. There were no relationships between the studied genes and histo-

pathological findings (*H. pylori* density, neutrophil activity, lymphoid aggregation in lamina propria and glandular atrophy). The strains which carry *cagA*, *vacAs1/m1*, *iceA2* and *babA* genes showed significant associations with severe active chronic gastritis ($P = 0.011$, 0.025 , 0.020 and 0.031 , respectively). The *vacAs1* genotype had significant correlation with the presence of the *cagA* gene ($P = 0.013$). Also, *babA* genotype showed associations with *cagA* ($P = 0.024$). In the combined genotypes, only *cagA*⁺/*vacAs1/m1*/*iceA2*/*babA*⁺ genotype showed correlation with severe active chronic gastritis ($P = 0.025$).

CONCLUSION: This genotyping panel can be a useful tool for detection of virulent *H. pylori* isolates and can provide valuable guidance for prediction of the clinical outcomes.

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Key words: *Helicobacter pylori*; *cagA*; *vacA*; *iceA*; *babA*

Vaziri F, Najar Peerayeh S, Alebouyeh M, Mirzaei T, Yamaoka Y, Molaie M, Maghsoudi N, Zali MR. Diversity of *Helicobacter pylori* genotypes in Iranian patients with different gastroduodenal disorders. *World J Gastroenterol* 2013; 19(34): 5685-5692 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v19/i34/5685.htm> DOI: <http://dx.doi.org/10.3748/wjg.v19.i34.5685>

INTRODUCTION

Infection with *Helicobacter pylori* (*H. pylori*) causes different clinical disorders such as persistent gastritis, peptic ulcers and mucosa associated lymphoid tissue (MALT) lymphoma. Current studies suggest that *H. pylori* infection may be a crucial risk factor in the development of gastric cancer^[1,2]. In this regard, this pathogen has been categorized as a group I carcinogen by the International Agency for Research on Cancer^[3]. The detailed reasons for these different clinical outcomes are unknown, but they may be related to host genetic factors, exposure to environmental factors (*e.g.*, diet, drug usage, acidity of the stomach and smoking) and to the bacterial genotypes^[4]. *H. pylori* shows extensive genetic diversity and this variability has a crucial role in pathogenesis of this bacterium^[5]. Several *H. pylori* virulence factor genes related to the risk of gastroduodenal disorders, including *cagA*, *vacA*, *babA* and *iceA*, have been proposed^[6]. A tremendous number of studies have proved that CagA and VacA producing strains are related to severe clinical outcomes^[7]. In addition to *cagA* and *vacA*, the other *H. pylori* virulence factors, such as *iceA* and *babA*, also showed such associations in some studies^[8,9]. Beyond the role of these factors in progression of the disease, there are several papers which reported a relationship between failure of *H. pylori* eradication therapy and the strains' virulence factor genotypes^[10]. Analysis of genetic structure of virulence factors among the isolates from different geographic regions will provide new insights regarding the pathogenesis and treatment of *H.*

pylori infection. *H. pylori* genotyping may have multiple roles including impact on the cure rates of eradication therapy^[10], determination of clinical outcomes^[11], tracking human migration^[12,13] and recently, the prediction of progression of gastric preneoplastic lesions^[14]. The distribution pattern of *H. pylori* genotypes and its correlation with disease outcome shows geographic differences. The aim of this study was to assess the diversity of *H. pylori* genotypes in an Iranian population to determine genotypically the *H. pylori* isolates more associated with different gastroduodenal disorders.

MATERIALS AND METHODS

Clinical specimens

Three gastric biopsies (two were used for histological examination and one for culture) were obtained from 177 adult patients undergoing routine diagnostic endoscopy referred to the Endoscopy Centre of Taleghani Hospital of Tehran, Iran, after obtaining informed consent. All subjects answered questionnaires related to age, sex, gastric or duodenal peptic ulcer diseases upon endoscopy.

Culture

Antral or body biopsy specimens from each patient were kept in transport medium consisting of thioglycolate with 1.3 g/L agar (Merck) and 3% yeast extract (Oxoid). The endoscopic biopsy specimens were cut into small pieces, homogenized with a sterile scalpel and were smeared on the surface of Brucella agar plates supplemented with 7% horse blood and Campylobacter selective supplement (vancomycin 2.0 mg, polymyxin 0.05 mg, trimethoprim 1.0 mg) and amphotericin B (2.5 mg/L). Incubation was performed in microaerophilic conditions at 37 °C for 5-7 d. Identification of *H. pylori* isolates was performed by analyzing colony morphology, Gram staining, oxidase, catalase and urease activities and *H. pylori*-specific polymerase chain reaction (PCR) (*glmM*). The isolates were preserved in BHI broth containing 20% glycerol and 10% fetal calf serum and stored at -70 °C.

DNA extraction

Genomic DNA was extracted with the QIAamp DNA mini kit (Qiagen, Germany) according to the manufacturer's instructions. The DNA was stored at -20 °C until used for molecular studies.

H. pylori genotyping

After DNA extraction, polymerase chain reactions (PCR) were performed in a volume of 25 µL containing 1 × PCR buffer, 1 µmol/L of each primer, 1 µL of genomic DNA (approximately 150 ng), 200 µmol/L of dNTPs mix, 2 mmol/L of MgCl₂, and 0.05 U/µL Taq DNA polymerase. PCR amplifications were performed in an automated thermal cycler (AG 22331; Eppendorf, Hamburg, Germany) under the following conditions: for *vacA s/m*: 33 cycles of 1 min at 94 °C, 33 s at 55 °C, and 1 min at 72 °C; for *cagA*: 33 cycles of 1 min at 94 °C, 1 min at

Table 1 Primers used in this study

| Gene | Primers (5'→3') | PCR product (bp) | Annealing temperature (°C) | Ref. |
|---|---|-------------------|----------------------------|------|
| <i>vacA</i> (s ₁ /s ₂) | VA1F: ATGGAAATACAACAAACACAc VA1R: CTGCTTGAATGCGCCAAAC | 259-286 | 55 | [6] |
| <i>vacA</i> (m ₁ /m ₂) | VACm1m2F: CAATCTGTCCAATCAAGCGAG VACm1m2R: GCGTCAAAAATAATTCCAAGG | 567-642 | 55 | [15] |
| <i>cagA</i> | CagAF: AATACACCAACGCCTCCAAG CagAR: TTGTTGCCGCTTTIGCTCTC | 400 | 59 | [16] |
| <i>iceA</i> ₁ | iceA1F: TATTTCTGGAACCTTGCCAACCTGAT M.Hpy1R: GGCCTACAACCGCATGGATAT | approximately 900 | 58 | [17] |
| <i>iceA</i> ₂ | iceA2 F: CGGCTGTAGGCACTAAAAGCTA iceA2 R: TCAATCCTATGTGAAACAATGATCGTT | approximately 800 | 58 | [17] |
| <i>babA</i> | babAF: CCAAACGAAACAAAAAGCGT babAR: GCTTGTGTAAGCCGTCGT | 271 | 58 | [18] |
| <i>glmM</i> | GlmM2-F GGATAAGCTTTTAGGGGTGTAGGGG GlmM1-R GCTTACTTTCTAACACTAACCGCGC | 296 | 52 | [19] |

59 °C, and 1 min at 72 °C; for *iceA*₁/*A*₂: 33 cycles of 1 min at 94 °C, 40 s at 58 °C, and 1 min at 72 °C, and for *babA*: 35 cycles of 1 min at 94 °C, 40 s at 58 °C and 1 min at 72 °C. The amplified genes were detected by electrophoresis in a 1.2% agarose gel with ethidium bromide. Table 1 summarizes the primer sequences, annealing temperatures and the expected size of the PCR products.

Histopathological evaluation

Sections were stained with hematoxylin and eosin for analysis of *H. pylori*-related histology by an expert pathologist. Then the grade of gastritis was scored based on the updated Sydney System.

Statistical analysis

Data were analyzed by using SPSS version 19.0.0 software (IBM, IL, United States). χ^2 test and Fisher's exact test were used to assess relationships between categorical variables. The level of statistical significance was set at $P < 0.05$.

RESULTS

Infection rates and clinical disorders

A total of 71 isolates from 177 patients (parenthesis approximately 40%) with different gastroduodenal disorders were obtained. The *H. pylori*-positive patients consisted of 24 males and 47 females, with their ages ranging between 19 and 85 years (mean age, 66 years). All of the isolates showed positive results for the common identification test and *H. pylori*-specific PCR (*glmM*). Most of the infected patients suffered from chronic gastritis (84.6%), while the others showed duodenitis (9.8%), intestinal metaplasia (2.8%), hyperplasia (1.4%) and gastric cancer diseases (1.4%) (Table 2).

Allelic diversities in main putative virulence markers

***cagA* genotyping:** The 400-bp PCR product indicating the presence of the *cagA* gene was obtained in 44 isolates (62%) and 27 (38%) were negative. Interestingly, evaluation of PCR results for *cagA* in 6 patients showed simultaneous existence of *cagA* variants according to their size

diversities.

***vacA* genotyping:** The frequency of *vacA* s₁, *vacA* s₂, *vacA* m₁ and *vacA* m₂ were 78.9%, 19.7%, 21.1% and 78.9%, respectively. Only one isolate was *vacA* som2 (with no PCR product for s region).

***iceA* genotyping:** Sole existence of *iceA*₁ genotype was detected in 15.5% and *iceA*₂ genotype in 22.5% of the colonized patients. Interestingly, out of the total studied samples, 40.8% were infected with both *iceA*₁ and *iceA*₂ genotypes and 21.1% were negative for these genes.

***babA* genotyping:** *babA* was found in 68 of the patients (95.8%); however, three patients (4.2%) did not show this allelic variant (Figure 1).

Correlation of *H. pylori* genotypes with pathological data, patients' age and clinical outcome

Combination of genotypes: Based on the analysis of the *cagA* gene (positive or negative), *vacA* s-region (s₁ or s₂), *vacA* m-region (m₁ or m₂), *iceA* allelic types (*iceA*₁ and *iceA*₂) and *babA* (positive or negative), twenty different genotypic combinations were recognized. The most prevalent genotype in *cagA* positive isolates was *cagA*⁺/*vacA*s₁m₂/*iceA*₁+*A*₂+/*babA*+ and in *cagA* negative isolates was *cagA*⁻/*vacA*s₁m₂/*iceA*-/*babA*+ (Figure 2).

***Helicobacter pylori* density, neutrophil activity, lymphoid aggregation in lamina propria and glandular atrophy:** There was no significant relationship between *cagA* positivity and *H. pylori* density, neutrophil activity, lymphoid aggregation in lamina propria and glandular atrophy in the biopsies. Also no relationships were found between other genes and these histopathological findings.

Patients' age: There was no significant relationship between the genotypes, clinical and pathological data and patients' age.

Chronic gastritis: The gastritis was scored as severe ac-

Table 2 Association of combined genotypes with pathological conditions in *Helicobacter pylori* isolates

| Combination of genotypes | SCG | SACG ² | MACG | MiACG | MCG | H | M | GC | D | Total | P value ¹ |
|--|-----|-------------------|------|-------|-----|---|---|----|---|-------|----------------------|
| <i>cagA</i> ⁺ / <i>vacAs1m2</i> / <i>iceA1+iceA2</i> / <i>babA</i> ⁺ | 1 | 12 | 2 | 0 | 0 | 0 | 1 | 0 | 1 | 17 | 0.025 ² |
| <i>cagA</i> ⁺ / <i>vacAs1m1</i> / <i>iceA2</i> / <i>babA</i> ⁺ | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 4 | |
| <i>cagA</i> ⁺ / <i>vacAs1m2</i> / <i>iceA1</i> / <i>babA</i> ⁺ | 0 | 3 | 1 | 0 | 0 | 0 | 1 | 0 | 2 | 7 | |
| <i>cagA</i> ⁺ / <i>vacAs1m1</i> / <i>iceA1+iceA2</i> / <i>babA</i> ⁺ | 0 | 6 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 8 | |
| <i>cagA</i> ⁺ / <i>vacAs2m2</i> / <i>iceA1+iceA2</i> / <i>babA</i> ⁺ | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | |
| <i>cagA</i> ⁺ / <i>vacAs0m2</i> / <i>iceA2</i> / <i>babA</i> ⁺ | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | |
| <i>cagA</i> ⁺ / <i>vacAs1m2</i> / <i>iceA2</i> / <i>babA</i> ⁺ | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 2 | |
| <i>cagA</i> ⁺ / <i>vacAs2m2</i> / <i>iceA1</i> / <i>babA</i> ⁺ | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | |
| <i>cagA</i> ⁺ / <i>vacAs2m2</i> / <i>iceA-</i> / <i>babA</i> ⁺ | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | |
| <i>cagA</i> ⁺ / <i>vacAs1m2</i> / <i>iceA-</i> / <i>babA</i> ⁺ | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | |
| <i>cagA</i> ⁺ / <i>vacAs1m2</i> / <i>iceA-</i> / <i>babA</i> ⁺ | 0 | 3 | 2 | 0 | 1 | 0 | 0 | 0 | 1 | 7 | |
| <i>cagA</i> ⁺ / <i>vacAs1m1</i> / <i>iceA-</i> / <i>babA</i> ⁺ | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 2 | |
| <i>cagA</i> ⁺ / <i>vacAs2m2</i> / <i>iceA2</i> / <i>babA</i> ⁺ | 1 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 3 | |
| <i>cagA</i> ⁺ / <i>vacAs1m2</i> / <i>iceA2</i> / <i>babA</i> ⁺ | 0 | 2 | 2 | 0 | 0 | 1 | 0 | 0 | 0 | 5 | |
| <i>cagA</i> ⁺ / <i>vacAs2m2</i> / <i>iceA1</i> / <i>babA</i> ⁺ | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | |
| <i>cagA</i> ⁺ / <i>vacAs1m1</i> / <i>iceA2</i> / <i>babA</i> ⁺ | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | |
| <i>cagA</i> ⁺ / <i>vacAs2m2</i> / <i>iceA-</i> / <i>babA</i> ⁺ | 0 | 3 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 4 | |
| <i>cagA</i> ⁺ / <i>vacAs1m2</i> / <i>iceA1+iceA2</i> / <i>babA</i> ⁺ | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | |
| <i>cagA</i> ⁺ / <i>vacAs1m2</i> / <i>iceA1+iceA2</i> / <i>babA</i> ⁻ | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | |
| <i>cagA</i> ⁺ / <i>vacAs2m2</i> / <i>iceA1</i> / <i>babA</i> ⁻ | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 2 | |
| Total | 2 | 39 | 13 | 1 | 5 | 1 | 2 | 1 | 7 | 71 | |

¹Only $P < 0.05$ are indicated; ²This P value is related to severe active chronic gastritis (SACG). SCG: Severe chronic gastritis; MACG: Moderate active chronic gastritis; MiACG: Mild active chronic gastritis; MCG: Moderate chronic gastritis; H: Hyperplasia; M: Metaplasia; GC: Gastric cancer; D: Duodenitis.

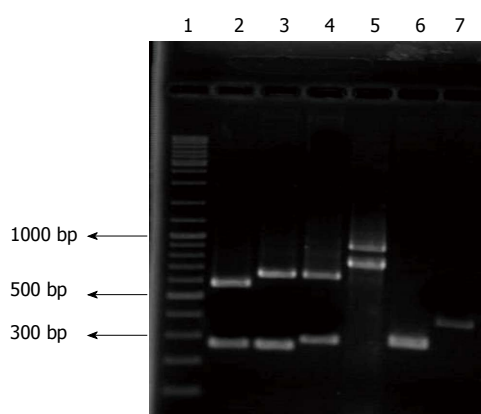


Figure 1 Polymerase chain reaction products of the main putative virulence markers. Lane 1: DNA ladder mix; Lane 2: *vacAs1m1* genotype; Lane 3: *vacAs1m2* genotype; Lane 4: *vacAs2m2* genotype; Lane 5: *iceA1+iceA2* genotype; Lane 6: *babA* genotype; Lane 7: *cagA* genotype

tive chronic gastritis, moderate active chronic gastritis, mild active chronic gastritis, severe chronic gastritis and moderate chronic gastritis. The strains which carried the *cagA* gene showed significant associations with severe active chronic gastritis ($P = 0.011$). Also, the strains which carried the *vacA* *s1/m1* gene showed significant associations with severe active chronic gastritis ($P = 0.025$). *babA* ($P = 0.031$) and *iceA2* ($P = 0.020$) also had significant correlation with severe active chronic gastritis. In the combined genotypes this association was observed for *cagA*⁺/*vacAs1m1*/*iceA2*/*babA*⁺ genotype in the case of severe active chronic gastritis ($P = 0.025$).

Genotype correlation: Interestingly, the *vacA* *s1* geno-

type had significant correlation with the presence of the *cagA* gene ($P = 0.013$). Also *babA* genotype showed this association in *cagA* positive isolates ($P = 0.024$).

DISCUSSION

H. pylori infection is usually present in 60%-80% of gastric and 95% of duodenal ulcers. However, some conditions affect infection rate of this bacterium in different geographic and socioeconomic regions. The prevalence of infection is typically higher in developing countries (greater than 80%) and lower in the developed ones (typically less than 40%)^[20]. It has been demonstrated that the prevalence of *H. pylori* infection in developing countries with low socioeconomic status and poor management of drinking water is much higher (> 80%) than that in developed countries (< 60%)^[21]. In our study the recovery rate of *H. pylori* was 40% which shows the improvement in the living conditions and hygiene in Iran that has also been reported recently^[22].

H. pylori can be divided into *cagA*-positive and *cagA*-negative strains, and there is increasing evidence that infection with *cagA*-positive isolates is associated with a greater risk of adverse clinical outcomes than infections with strains lacking this gene. In the current study, the strains which carried the *cagA* gene showed significant associations with severe active chronic gastritis. Interestingly, the prevalence of the *cagA*-positive strain differs among different countries, and more than 90% of *H. pylori* strains are *cagA*-positive in East Asian countries, irrespective of clinical presentation^[23]. Sasaki *et al*^[24] showed that among *H. pylori* DNA-positive samples, *cagA* was detected in 45.9% from Ecuador and 20.0% from Panama.

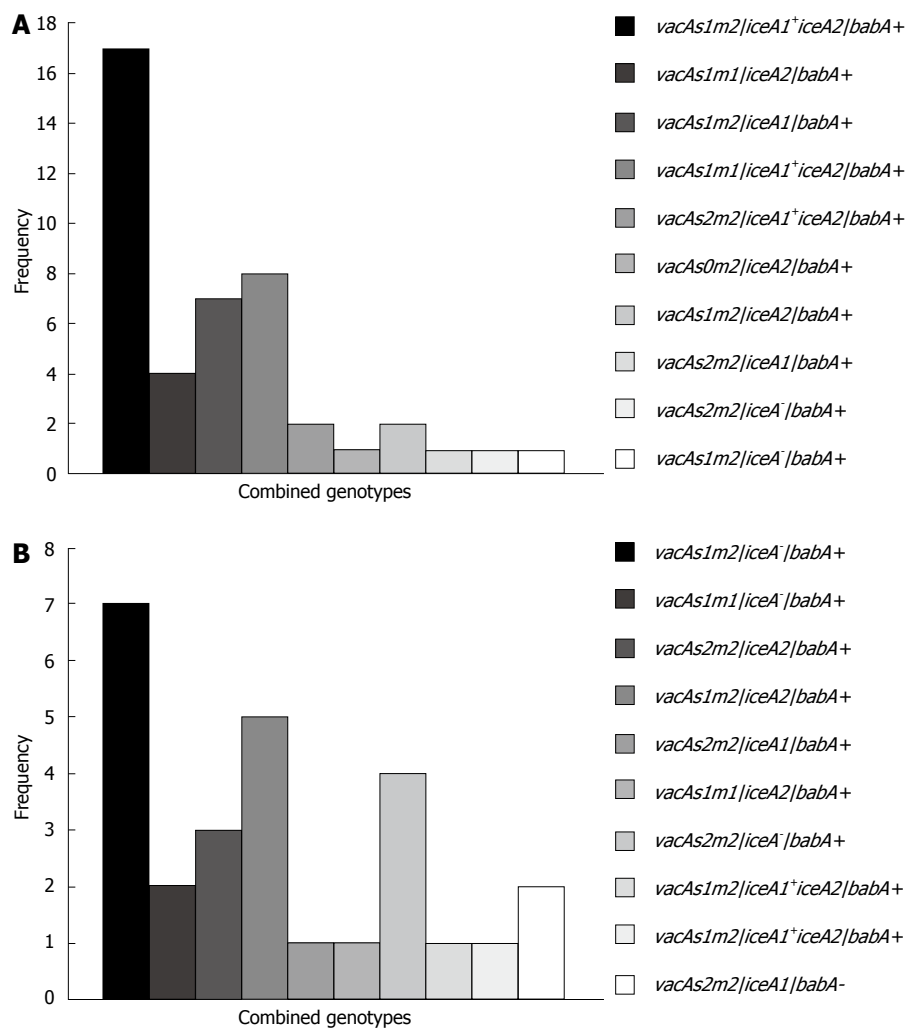


Figure 2 The frequency of combined genotypes. A: Combined *vacA*, *iceA* and *babA* genotypes in 44 *cagA* positive isolate; B: Combined *vacA*, *iceA* and *babA* genotypes in 27 *cagA* negative isolates.

In our study the prevalence of *cagA*-positive isolates is 62% which is less than other Asian countries and more than other countries (*e.g.*, Ecuador, Panama). According to Watada *et al.*^[25] study, the prevalence of *cagA* was 65.5% in Colombia and 100% in Japan, which showed that the prevalence of this gene in our study is similar to the Colombian isolates. In another study conducted in Bulgaria, the prevalence of *cagA* was 84.9% which is more than our results^[26]. Interestingly, we had 6 isolates which had two different sizes of *cagA* simultaneously, showing the occurrence of mixed infection in these patients.

Variations of *vacA* are associated with different risks of gastrointestinal disorders. In general, *vacA* *s1* and *m1* genotypes produce a large amount of toxin, whereas *s2* and *m2* genotypes show little or no toxin production^[27]. Recently, a third polymorphic determinant of vacuolating activity has been described as located between the s-region and m-region, an intermediate (i) region^[28]. The frequency of the *vacA* *s1* and *vacA* *m1* genotypes in the Middle Eastern countries was found to be 71.5% and 32.8%, respectively^[11], which is in concordance with our study. We did not detect any *vacA* *s2m1* genotypes in our isolates which

has been reported to be rare^[23]. The *vacA* *s1* and *m1* genotypes have been reported to be associated with *H. pylori*-related diseases; however *vacA* *s2* and *m2* strains are rarely associated with peptic ulcer and gastric cancer because of their low or non-vacuolating activities^[23]. Genotyping of *vacA* will be useful in screening individuals for risk factors associated with gastric cancer and peptic ulcer development. Asrat *et al.*^[29] showed that *vacAs1m1* genotype was the most common genotype in Ethiopian adult dyspeptic patients, and also *vacA*- and *cagA*-positive *H. pylori* strains were detected to a higher degree in patients with chronic active gastritis. Interestingly, similar to our results, correlation of the *vacA* *s1* genotype with the presence of the *cagA* gene was reported by Atherton^[30]. The *vacAs1m2* genotype is more common in our Iranian patients, as previously described in Iran^[31]. As reviewed by Suzuki *et al.*^[32], the predominant *vacA* genotypes in Asia, Europe and Africa are *vacAs1m1* and their subtypes, which is in contrast to our genotypes in Iranian isolates.

In spite of the low frequency of *vacAs1m1* genotypes in our study, isolates which carried the *vacAs1m1* gene showed significant associations with severe active chronic

gastritis. In a review by Hosseini *et al*^[33], they concluded that in contrast to *vacA*, there is no correlation between *cagA* genotype and disease status in the majority of studies conducted in Iran; but results of our study, however, proposed both of these genetic markers as useful indicators for predicting clinical outcomes in the studied population.

The meta-analysis by Shiota *et al*^[8] confirmed the importance of the presence of *iceA* gene for peptic ulcer, although the significance was controversial. Such different results between the *iceA* allelic types and clinical disorders could be explained by the difference in geographic regions. In our study we found a significant relationship between *iceA*₂ genotype and clinical outcomes (severe active chronic gastritis), which was also observed by Caner *et al*^[34] in Turkey. As Shiota *et al*^[8] summarized in their meta-analysis, most of the studies showed no association between *iceA*₁ and *cagA* status, which is in concordance with our study. Interestingly, the prevalence of mixed genotype *iceA*₁ + *iceA*₂ (40.8%) in our study was higher than other studies which had detected this mixed genotype^[35-37]. So this high prevalence with mixed genotypes makes it difficult to analyze potential relationships between the presence of each *iceA* allelic variant and clinical outcomes. *babA* genotype was frequently found in *H. pylori* strains in our study (95.8%); this was associated with severe active chronic gastritis. Although this genotype showed significant correlation with the existence of *cagA*, no significant correlation was observed with other virulence factors such as *vacA* *s*₁/*s*₂, *vacA* *m*₁/*m*₂ and *iceA*₁/*iceA*₂. Chomvarin *et al*^[38] detected the *babA* gene in 92% (103/112) of Thai patients, which is almost similar to our results; while in another study conducted in Cuba the prevalence of *babA* gene was lower (82.3%)^[39]. It is important to mention that this PCR based method for *babA* genotyping must be confirmed by immunoblotting. Actually isolates were scored as *babA*-gene positive if the PCR and/or Southern blot analysis yielded a positive result^[9].

Regarding the combination of genotypes, we observed twenty different genotypes which showed vast diversities in the *H. pylori* isolates in our study. Interestingly there was not any significant association between these combined genotypes and clinical outcomes, except for *cagA*⁺/*vacA*₁*m*₁/*iceA*₂/*babA*⁺ genotype which showed significant association with severe active chronic gastritis.

Genotypes of *H. pylori*, especially *cagA* and *vacA*, are reported to be crucial factors determining the cure rates. So to select an *H. pylori* eradication regimen, we need to consider *H. pylori* genotypes^[10]. *H. pylori* genotype distributions and their correlations with disease outcomes have shown geographical differences. In this regard, Yamaoka *et al*^[7] reviewed that within East Asia, where the incidence of gastric cancer is high, that *vacA* *m*₁ genotype is dominant; whereas in southern parts where the gastric cancer incidence is low, the *m*₂ genotype, which we observed in our study, is predominant. Dabiri *et al*^[31] showed that there was statistically no association between the *vacA*,

cagA and *cagE* status and clinical outcomes in Iranian patients, and recommended that other different markers may be more useful for this analysis. In comparison, in the current study, genotyping on the basis of *cagA*, *babA*, *vacA* and *iceA* was considered as a useful tool for predicting the clinical outcomes. Therefore, analyzing the multiple virulence factors of *H. pylori* (*cagA*, *vacA*, *iceA* and *babA*) might enable us to predict the patient's clinical outcome among Iranian patients. This prediction could be more accurate when accompanied by the impacts of environmental factors and host genetic polymorphisms such as interleukin-1 receptor antagonist gene polymorphism^[37]. Nowadays, concurrent genotyping of *H. pylori* virulence markers and host factors is becoming increasingly crucial in the prediction of the diseases outcomes^[40].

In conclusion, our results show that most of the *H. pylori* isolates were highly virulent on the basis of the main clinically allelic variants in three or four virulence factors they carried. The Iranian isolates predominantly possessed different genotypes which showed vast diversities. Significant association of the noted genotypes with severe active chronic gastritis suggests that this genotyping panel is a suitable tool for detection of virulent *H. pylori* isolates that could provide valuable guidance for prediction of the clinical outcomes.

ACKNOWLEDGMENTS

The authors would like to thank Leila Shokrzadeh and Ehsan Nazemalhosseini from the Gastroenterology and Liver Diseases Research Center, Shahid Beheshti University of Medical Sciences.

COMMENTS

Background

Infection with *Helicobacter pylori* (*H. pylori*) causes diverse clinical outcomes such as persistent gastritis, peptic ulcers, mucosa associated lymphoid tissue lymphoma and gastric cancer. One of the reasons for these different clinical outcomes is genetic diversity of *H. pylori*; therefore determination of the pattern of *H. pylori* genotypes and its correlation with disease outcome, which shows geographic differences, is crucial.

Research frontiers

The *H. pylori* genotyping may have multiple roles including prediction of clinical outcomes, impact on the *H. pylori* infection therapy, tracking human migration, and recently, the prediction of progression of gastric preneoplastic lesions. Therefore genotyping of *H. pylori* can be a valuable and multifunctional tool in the clinical field.

Innovations and breakthroughs

In the majority of previous studies, the researchers were not able to detect any significant relationship between their genotyping panels and clinical outcomes for *H. pylori* infections. Most of these studies had used few genetic markers. In order to overcome this disadvantage, the authors have chosen greater numbers of *H. pylori* genetic markers for studying this association.

Applications

The genotyping panel which contains eight important genetic markers can serve as a useful tool for typing of *H. pylori* isolates and, to some extent, predict clinical outcomes.

Peer review

This is an epidemiological paper with statistical analysis, dealing with the important question of association between certain *H. pylori* genotypes and specific

pathologies, and with the problem of predictive value of *H. pylori* infection genotyping. In the submitted manuscript this issue is dissected in fine detail and uses quite extensive clinical material, thus providing novel and more reliable data.

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ISSN 1007-9327



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