Original Article

Prevalence of *Helicobacter pylori vacA* different genotypes in Isfahan, Iran

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Abstract

Background: It is believed that the *Helicobacter pylori* (*H. pylori*) *vacA* gene, as a major virulence determinant (One of the major virulence determinant, not major), may be a risk factor for the development of gastroduodenal diseases. The frequency of *vacA* genotypes varies in different human populations. This study evaluated the prevalence of *vacA* alleles/genotypes among dyspeptic patients in Isfahan.

Materials and Methods: One-hundred *H. pylori*-positive adult patients were examined in this study. After culture of gastric biopsies and DNA extraction from individual *H. pylori* isolates, the (all *H. pylori* strains harbor vacA alleles, please replace "presence" with "genotypes") of the vacA s and m alleles were determined using polymerase chain reaction (PCR).

Results: There were four vacA mosaicisms, including 28 for s1a/m1 (28%), 23 for s1b/m1 (23%), 26 for s1a/m2 (26%) and 23 for s1b/m2 (23%). The s2 allele was not found. The predominant vacA genotype in patients with non-ulcer dyspepsia and duodenal ulcer was s1a/m2, whereas in patients with adenocarcinoma was s1a/m1.

Conclusion: The results showed there was no significant correlation between different genotypes of the *vacA* and the clinical outcomes and appears to *vacA* genotypes were not useful determinants for gastrointestinal diseases in our area.

Key Words: Adenocarsinoma, gastroduodenal diseases, *Helicobacter pylori*, Iran, Peptic ulcere, *vacA* gene

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INTRODUCTION

Helicobacter pylori is a gram-negative, curved and microaerophilic bacterium. It infects the stomach mucosa of more than 50% of the world's human populations.[1-3] It may cause variety of clinical symptoms such as chronic gastric inflammation, peptic ulcer disease (PUD), MALT lymphoma and gastric adenocarcinoma.[4,5] The majority of *H. pylori* infected persons are asymptomatic, and only a fraction

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(10-20%) of carriers' manifest clinical disease.[6,7] The prevalence of *H. pylori* infection is variable in different countries.[3] In Iran as a developing country, the rate of infection has been reported to be 60-90%.[8-10]

Some virulence-related genes such as *vacA* and *cagA* in the clinical isolates have been previously studied, however, the role of *H. pylori* isolates genotypes in different symptoms remains controversial.[11] The vacuolating-cytotoxin (VacA) is one of the major virulence factors, which encoded by the *vacA* gene.[12,13] This 87 kDa protein induces both vacuole formation and apoptosis in gastric epitheliums.[14,15] Although the *vacA* gene is present in all *H. pylori* strains, however, approximately 30-66% of clinical isolates produce the VacA cytotoxin.[4,16] The *vacA* gene contains two variable parts, the s region, and the m region, which encode the signal, and middle peptides, respectively.[17] The s region consisting of s1 (subtypes s1a, s1b and s1c) and s2 alleles, and m region also includes m1 (subtypes m1a, m1b and m1c) and m2 alleles, respectively.[14,18] The *vacA* s1 and m1 alleles have greater cytotoxic activity than s2 and m2 alleles.[19] A novel polymorphism in the intermediate (i) region (contain i1 and i2 alleles) between the s and m areas. They found that the i1 allele in Iran is significantly associated with gastric cancer.[20] The existence of different genotypes of *vacA* (s1/m1, s1/m2, s2/m1 and s2/m2) is due to various possible combinations of s and m alleles, that are virulence-associated.[18,21] Among these genotypes, the frequency of *vacA* s2/m1 is rare.[22] *H. pylori* s1/m1 genotypes offer the high levels of cytotoxic activity, and are associated to inflammation and ulcer[18,23] whereas, s1/m2 strains produce moderate amounts of toxin, and s2/m2 strains produce very little or no toxin.[14,15,18]

The correlation between the *vacA* genotypes and gastroduodenal manifestations are variable in terms of geographic areas. On the other hand, the distinct dominant *vacA* genotypes differ among clinical isolates from the various geographic regions,[13] because of the lack any report in the case of *H. pylori vacA* genotypes in Isfahan (center of Iran); therefore, to understand the clinical relevance of *H. pylori* genotyping in predicting infection outcome, we carried out this study in order to survey prevalence of *vacA* genotypes, and their relevance with different gastroduodenal diseases.

MATERIALS AND METHODS

Patients and clinical samples

H. pylori strains, isolated from 100 adult patients of both sexes with gastroduodenal manifestations at the Department of Gastroenterology of Hospital Al-Zahra in Isfahan, Iran, were included. All patients underwent upper gastrointestinal endoscopy for both visual examination and biopsy collection. Exclusion criteria were anti-*H. Pylori* therapy intake in the last 4 weeks. Based on endoscopic examination and clinical signs, patients were classified into the following groups: non-ulcer dyspepsia (n = 40), duodenal ulcer (n = 40), and adenocarcinoma (n = 20). From each patient, three antrum biopsy samples were taken for rapid urease test (RUT), histology and culture.

Culture and identification of *H. pylori*

For bacterial culture, biopsy specimens were processed according to the method of Lang and Garcia.[24] Plates were incubated in microaerobic conditions (5% O_2, 10% CO_2 and 85% N_2) in anaerobic jars (MART, ANOXOMAT, Lichtenvoorde, the Netherlands) at 37°C for 3-5 days. Colonies were identified as *H. pylori* based on biochemical tests. All the isolates were stored at –80°C in aliquots of BHI broth (Merck, Germany) supplemented with 20% (v/v) glycerol.

DNA extraction

For each isolate, extraction of chromosomal DNA was performed using phenol-chloroform method.[25] Briefly, *H. pylori* cells were washed twice in 1000 µl TE buffer (10 mM Tris, 1 mM EDTA, pH 8.0), then the pelletes were resuspended in 500 µl TE buffer and 100 µl lysozyme (10 mg/ml), and then lysed using SDS – proteinase K at 56°C for 1 h. The cell extract was then treated with phenol/chloroform isoamylalcohol, ethanol precipitation for DNA extraction.

Polymerase chain reaction

The primers was chosen for *H. pylori vacA* evaluation, are listed in Table 1. PCR was performed as described in previous references.[17]

Data analysis

The association between *H. pylori* genotypes and clinical relevance was analyzed by Pearson Chi-square or Fisher's exact *test* (when necessary). P value <0.05 was considered statistically significant.

Table 1: Oligonucleotide primers used for *H. pylori* vacA alleles

Gene	Primer designation	Primer sequence	PCR product size
m1	VA3-F	5′ GGTCAAAATGCGGTCATGG 3′	290 bp
	VA3-R	5′ CCATTGGTACCTGTAGAAAC 3′	
m2	VA4-F	5′ GGAGCCCCAGGAAACATTG 3′	352 bp
	VA4-R	5′ CATAACTAGCGCCTTGCAC 3′	
s1a	SS1-F	5′ GTCAGCATCACACCGCAAC 3′	190 bp
	VA1-R	5′ CTGCTTGAATGCGCCAAAC 3′	
s1b	SS3-F	5′ AGCGCCATACCGCAAGAG 3′	187 bp
	VA1-R	5′ CTGCTTGAATGCGCCAAAC 3′	
s2	SS2-F	5′ GCTAACACGCCAAATGATCC 3′	199 bp
	VA1-R	5′ CTGCTTGAATGCGCCAAAC 3′	

RESULTS

Our population consisted of 100 adult patients (55 males and 45 females) ranging in age from <30 to >70 years (mean 43 years). The distribution of patients according to age and sex is shown in Table 2.

H. pylori vacA alleles and genotypes

Correlation between *vacA* subtypes and clinical diseases is shown in Table 3.

The most prevalent *vacA* allele in different samples was s1a (54%), m1 being present in 51%, followed by m2 and s1b with frequencies of 49 and 46%, respectively. Overall, 28% of isolates were grouped as *vacA* s1a/m1, 23% as s1b/m1, 26% as s1a/m2, and 23% as s1b/m2 genotypes. However, *vacA* s2 allele in none of patients was observed. The dominant *vacA* genotype in patients with NUD and DU was s1a/m2, whereas in patients with AC was s1a/m1. In our study, the prevalence of *H. pylori vacA* genotypes was not significantly associated with different clinical manifestations ($P > 0.005$).

DISCUSSION

H. pylori is strongly associated with gastritis and peptic ulcer.[12] The variable prevalence of infection caused by this bacterium in various countries is due to its variable clinical isolates.[16] Considering the necessity of *H. pylori* genotype analysis in different populations and various areas of the world, we conducted this study to evaluate the prevalence of *vacA* genotypes and its relationship to different clinical outcomes in of Isfahan. Our study shows there is no s2 allele in *H. pylori* genome in the area of Isfahan, whereas m1 and m2 alleles were found in the mid region and s1a and s1b alleles were present in the signal sequence of *H. pylori* genome. A study conducted by Van Doorn *et al.*[26] in 1998 reported the frequency of s1a/m1, s1a/m2, s1b/m1, s1b/m2 and s2/m2 to be 36, 23, 2, 5 and 20%, respectively. In our study these values were found to be 28, 26, 23 and 23%, respectively, which indicates the various genotype frequencies according to different geographical regions. A study performed by Strobel *et al.*[27] in Germany also indicates that 96% of the strains isolated in this country contain s1 gene while s2 and m1 genes account for 4% and 51% of the strains, respectively. In a study carried out in Turkey it was shown that 87.7% of the strains isolated from gastrointestinal patients (NUD, DU, GC) harboured s1 gene, 12.3% contained s2 gene and 43% contained m1 gene[28] indicating the similarity of the studies carried out in Turkey and Germany. A meta-analysis of 1646 patients in the Middle East reported the frequency of the genotypes s1/m1 and s1/m2 in the northern and southern countries of this region to be 34%, 14.8%, 44.8% and 30.1%, respectively(29). These data are significantly different from the results of our study. In a study performed in Thailand the frequency of the genotypes s1 and s2 was shown to be 100% and 0%. The allelic variant *vacA* s1/m1 was more prevalent than s1/m2. Different genes either single or in combination had no significant relationship with clinical diseases.[11] This is in accordance with the study carried out in Isfahan with the exception that there was no significant difference in the prevalence of the above mentioned genotypes in our study. In four studies carried out in Iran the prevalence of s1 allele as the most common allele of VacA cytotoxin subtypes was found to be 71%, 69%, 69% and 70.5%, respectively and is in accordance with our study.[30-33] But contrary to our study, s2 allele was present in all these studies. The dominant genotype in Jafari, Dabiri and Molaei studies was *vacA* s1/m2, whereas in Isfahan this prevalence was 51% for *vacA* s1/m1 and 49% for s1/m2. In a study carried out in Pakistan[15] the most prevalent genotype was found to be s1b/m2 followed by s1a/m1, whereas in our study s1b/m2 and s1b/m1 had the lowest frequencies. In the mentioned study the frequency of s1a/m1 and *cagA* was lower than those reported by other neighbouring countries. In a study performed in Costa Rica the frequency of the genotypes s1b and m1 was reported 75.2% and 74.2%, respectively. In this study a significant association was observed between m1 genotype

Table 2: Frequency of patients according to age and gender

	Clinical outcome			Total (No.) (%)
	AC (No.) (%)	DU (No.) (%)	NUD (No.) (%)	
Age (years)				
<30	0 (0)	9 (22)	5 (13)	14 (14)
31-49	2 (12)	15 (38)	24 (60)	41 (41)
50-70	8 (38)	13 (32)	7 (17)	28 (28)
>70	10 (50)	3 (8)	4 (10)	17 (17)
Gender				
male	12 (60)	26 (65)	20 (50)	58 (58)
Female	8 (40)	14 (35)	20 (50)	42 (42)

NUD: Non-ulcer dyspepsia, DU: Duodenal ulcer, AC: Adenocarcinoma

Table 3: Relationship between *H. pylori* vacA alleles/genotypes and clinical status

vacA allele/genotype	Clinical status			Total (No.) (%)
	AC (No.) (%)	DU (No.) (%)	NUD (No.) (%)	
s1a	9 (45)	23 (57.5)	22 (55)	54 (54)
s1b	11 (55)	17 (42.5)	18 (45)	46 (46)
m1	12 (60)	21 (52.5)	18 (45)	51 (51)
m2	8 (40)	19 (47.5)	22 (55)	49 (49)
s1a/m1	7 (35)	11 (27.5)	10 (25)	28 (28)
s1b/m1	5 (25)	10 (25)	8 (20)	23 (23)
s1a/m2	2 (10)	12 (30)	12 (30)	26 (26)
s1b/m2	6 (30)	7 (17.5)	10 (25)	23 (23)

NUD: Non-ulcer dyspepsia, DU: Duodenal ulcer, AC: Adenocarcinoma

and gastritis.[34] The frequency of genotypes s1b and m1 was 45% each in our work, which is a lower percentage compared to the above study, although no significant correlation was observed between genotype m1 and gastritis. In a study performed in Thailand 58 gastritis patients (Ga), 28 gastric ulcer (GU), 45 duodenal ulcer (DU) and 4 gastric cancer (GC) patients were analysed, which the frequency of s1a and s1a/m1 genotypes were reported 81% and 21%, respectively.[35] The frequency of s1a allele is lower in our study while the frequency of s1a/m1 genotype is similar (28%) to the above study. In a study conducted in Pakistan,[16] the frequency of s1a/m1 was found to be 69% which was much higher than our findings. In the mentioned study in Turkey has shown the frequency of s1/m1 and s1/m2 genotypes was 41% and 48%, respectively. They also reported the frequency of s1a allele in NUD, DU and GC cases as 67, 97 and 88%, respectively. In a survey carried out in Bahrain, all patients with ulcer and 82.4% of patients with NUD contained the s1 strains.[36] In the present study all patients with DU and NUD had strains containing the s1 genotype. In a study conducted by Boyanova *et al.*[6] patients with ulcer had a higher number of virulent strains compared to other patients. In our study patients with AC and DU had a higher number of strains containing the s1/m1 genotype compared to subjects with NUD (64.7% compared to 35.3%). In addition, in the mentioned study similar to ours, s2 allele was not found in patients with ulcer but was only found in 13% of other patients. No *vacA* s2 genotype was reported in the study carried out by Sheu *et al.*[13] In a study conducted by Ohno *et al.*[37] among several Asian and European countries the *vacA* genotypes was shown to be different in these countries and the s2/m2 genotype was not found in Asian patients similar to our findings, whereas this genotype was found in western patients. In a study by Chomvarin *et al.*[11] no significant relationship was detected between *vacA* genotypes and other virulence genes such as *iceA*, *cagA*, *babA2* and the complication produced in the patient, which confirms the findings in this report. These results were also reported in the previous studies carried out in Iran, Taiwan and Mexico. In the studies conducted by Yakoob, *et al.*,[16] Molaei, *et al.*,[33] Chiarini, *et al.*,[38] Bindayna and Mahmeed[36] significant association was found between *vacA* genotypes and clinical outcomes. Hussein *et al.*[19] also found such relationship between *vacA* genotypes and PUD in strains isolated from Iraqi patients, however, this relationship was not significant in the Iranian strains.

CONCLUSION

Considering the mentioned studies, it is safe to say that the frequency of the s2 genotype is low in most studies which are confirmed by the results in our study. Other genotypes such as s1a, s1b, m1 and m2 are significantly different in different regions which indicate the various distributions of these genotypes. Overall, it is understood that there is no correlation between *H. pylori vacA* genotype and the resulting disease. Therefore each of the *vacA* genotypes causing the complication may be found in different patients.

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