Helicobacter pylori hopQ alleles (type I and II) in gastric cancer

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Received December 22, 2015; Accepted March 2, 2016

DOI: 10.3892/br.2016.634

Abstract. The *Helicobacter pylori* (*H. pylori*) outer membrane protein (HopQ) of is one of the proteins involved in bacterial adherence to gastric mucosa and has been suggested to have a role in the virulence of *H. pylori*. The aim of the present study was to determine the association between *H. pylori* virulence types I and II *hopQ* genotypes and patients with different gastrointestinal diseases. A polymerase chain reaction-based assay was used to determine the presence of type I and type II *hopQ* genes in 88 *H. pylori* strains isolated from *H. pylori*-infected patients. Of the total 88 *H. pylori* isolates, type I and type II *hopQ* alleles were detected in 52 (59.1%) and 36 (40.9%), respectively. A significant association was found between type I *hopQ* gene and gastric cancer [odds ratio, 2.3; 95% confidence interval (CI), 1.3-4.1] and gastric ulcers (odds ratio, 2.5; 95% CI, 1.4-4.3). A significant association was also identified between the type II *hopQ* gene and gastric cancer (odds ratio, 2.4; 95% CI, 1.1-3.0). The association between *hopQ* type I and *hopQ* type II genotypes and clinical status suggest that these genes may be helpful in the universal prediction of specific disease risks.

Introduction

Helicobacter pylori (*H. pylori*) is a Gram-negative bacterium that persistently colonizes in the stomachs of more than half the human population (1). Colonization of the stomach by *H. pylori* consistently induces gastric inflammation, known as superficial chronic gastritis, and is a risk factor for the development of peptic ulcer disease and gastric malignancies (2). *H. pylori* strains are thought to possess various virulence factors, which contribute to digestive disease complications (3). In 2005, it was reported that *H. pylori hopQ* genotypes are associated with an increased risk for peptic ulcer disease (4). *H. pylori* genomes contain ~30 paralogous *hop* genes, which encode outer membrane proteins (5). HopQ is localized at the surface of *H. pylori* (6). The *H. pylori* outer membrane protein (Hop) family is the largest and includes adhesions such as BabA (HopS) (7), SabA (HopP) (8), OipA (HopH) (9), AlpAB (HopB and HopC) (10) and HopQ (11). However, nothing is known regarding the functional properties of HopQ and this study did not provide further data regarding disease-specific virulence factor of *hopQ*. *H. pylori hopQ* alleles exhibit a high level of genetic diversity, and two families of *hopQ* alleles have been described (type I *hopQ* and type II *hopQ*) (12). Iran is a developing country with a high prevalence of *H. pylori* infections, among symptomatic and asymptomatic individuals, and the prevalence is ≤90% in the northwestern part of the country (13). The aim of the present study was to determine the association between *H. pylori* virulence type I and type II *hopQ* genotypes and patients with different gastrointestinal diseases.

Materials and methods

Patients. In the study, patients undergoing upper gastric endoscopy due to different digestive diseases that visited the hospitals in Tabriz (Iran) were included. The standard number of gastric biopsy samples for patients who were suspected of being infected with *H. pylori* was obtained for routine culture and histological investigations. Patient groups according to endoscopic and pathology findings were: Gastric ulcer (GU), duodenal ulcer (DU), gastritis and gastric carcinoma (GC). Written informed consent was obtained from all the patients prior to entering the study, and the study was approved by the Regional Ethics Committee, Tabriz University of Medical Sciences (Tabriz, Iran; no. 5/47/1375, April 4, 2015).

H. pylori isolates and bacterial culture. The presence of *H. pylori* was determined by cultures placed on Brucella agar (Merck KGaA, Darmstadt, Germany) containing 5% sheep blood and antibiotics such as amphotericin B (5 mg/ml), trimethoprim lactate (5 mg/l), vancomycin (10 mg/ml) and polymixin-B (2,500 U/ml). Plates were incubated at 37˚C, in 10% CO_2 conditions, and were subsequently identified as *H. pylori*, based on: Colony morphology, Gram-staining and positive oxidase, catalase and urease tests. In the present study, the *H. pylori* American Type Culture Collection (ATCC) 43504 strain (ATCC, Manassas, VA, USA) was used as a reference.

DNA extraction and polymerase chain reaction (PCR). Bacterial DNA was extracted from single colonies of *H. pylori*

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Key words: Helicobacter pylori, virulence factor, outer membrane proteins

Table I. Amplification primers for amplification used in the study.

Study, year	DNA region amplified	Primers	Primer sequence	Refs.
Kidd *et al*, 1999	*glm*M	HP-F	AAGCTTTTAGGGGTGTTAGGGGTTT	(16)
		HP-R	AAGCTTACTTTCTAACACTAACGC	
Sicinschi *et al*, 2012	Type I *hopQ*	HP-F	CAACGATAATGGCACAAACT	(17)
		HP-R	GTCGTATCAATAACAGAAGTTG	
Sicinschi *et al*, 2012	Type II *hopQ*	HP-F	TCCAATCCAGAAGCGATTAA	(17)
		HP-R	GTTTTAATGGTTACTTCCACC	

Table II. Distribution of *Helibacter pylori hopQ* genes (type I and II) among the different groups.

Diseases (no.)	Rate of type I *hopQ*, no. (%)	Odd ratio (95% CI)	Rate of type II *hopQ*, no. (%)	Odds ratio (95% CI)
Gastric carcinoma (26)	18 (69.2)	2.3 (1.3-4.1)	16 (61.5)	2.4 (1.1-3.0)
Duodenal ulcer (16)	6 (37.5)	0.6 (0.3-1.0)	2 (12.5)	0.2 (0.1-0.4)
Gastric ulcer (10)	10 (100.0)	2.5 (1.4-4.3)	4 (40.0)	0.7 (0.4-1.2)
Gastritis (36)	18 (50.0)	Control group	14 (38.9)	Control group

HopQ, *Helibacter pylori* outer membrane protein; CI, confidence interval.

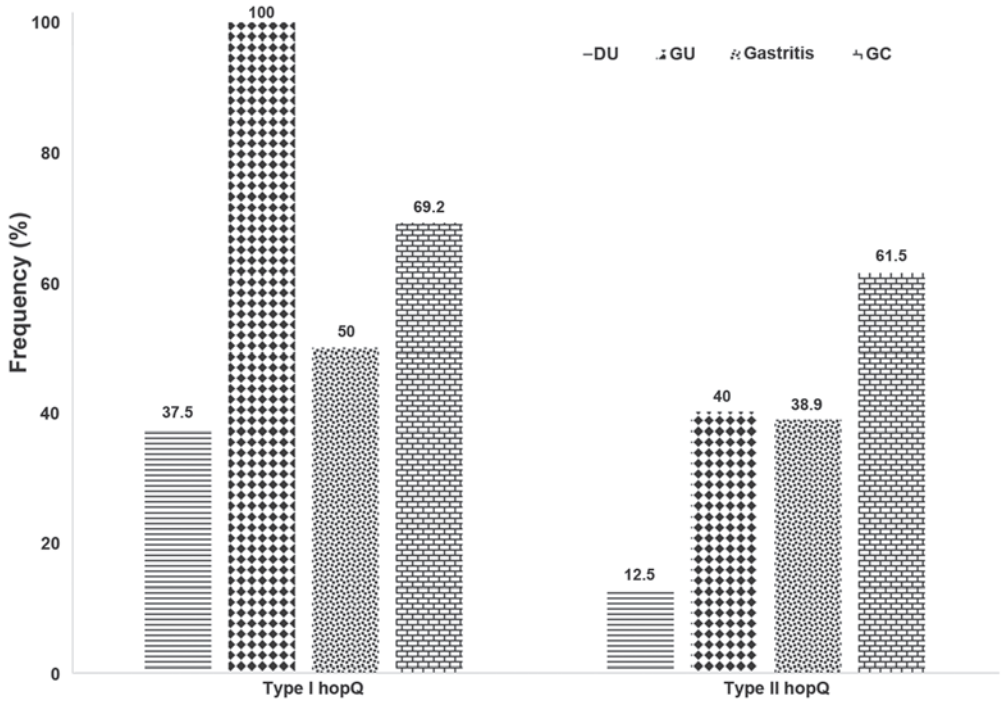

Figure 1. Frequency of hopQ types among different diseases.

and their DNA was extracted by sodium dodecyl sulphate, proteinase K and the cetyltrimethyl ammonium bromide method (14) and stored at -20˚C. Initially, the PCR assay was used to detect the *H. pylori*-specific *ure*C (*glm*M) gene for confirmation of *H. pylori* strains (15). In this experiment, *hopQ* type I and type II genes were detected by the PCR method (16,17) (Table I), under the following conditions for both genes: 35 cycles at 94˚C for 60 sec, at 54˚C for 45 sec, and at 72˚C for 65 sec, and a final extension at 72˚C for 10 min. PCR products were analyzed on 1.5% agarose and the strains containing the *hopQ* type I and *hopQ* type II genes were used as a positive control.

Statistical analysis. The data obtained were analyzed by SPSS (version 19; IBM, Corp., Armonk, NY, USA) and the χ^2 test and Fisher's exact test was used to compare the clinical

outcomes and the presence of genes. P<0.05 was considered to indicate a statistically significant difference.

Results

H. pylori culture positive. From 286 gastric biopsies, 88 (30.76%) were positive for *H. pylori*. Of the patients (including 88 *H. pylori*-positive) that attended the endoscopy ward, 6 suffered from anemia (6.8%), 24 from epigastria pain (27.3%), 10 from gastro-esophageal reflux disease (11.4%), 46 from dyspepsia (52.3%), and 2 from gastrointestinal bleeding (2.3%). According to endoscopy and pathology findings, among the remaining 88 *H. pylori* culture-positive patients, 10 (11.4%) had GU, 16 (18.2%) had DU, 36 (41.0%) had gastritis and 26 (29.5%) had GC (Fig. 1).

Using primers for the *ure*C (*glm*M) gene, the PCR product of 294-base pairs was obtained in all 88 strains. The mean age of patients was 40±15 years, ranging from 18 to 84 years and including 40 (45.5%) males and 48 (54.5%) females. The data showed that of 88 isolates, *hopQ* type I was present in 52 (59.1%) isolates and *hopQ* type II was found in 36 (40.9%) isolates. Table II depicts the prevalence of these genes in association with the different disease groups.

Associations between hopQ types and disease. There were no statistically significant associations between age and gender with *hopQ* types (P>0.05). Statistical analysis indicated significant associations between *hopQ* type I and GC [odds ratio, 2.3; 95% confidence interval (CI), 1.3-4.1] and GU (odds ratio, 2.5; 95% CI, 1.4-4.3). Type II *hopQ* was also found to be significantly predominant in patients with gastric cancer (odds ratio, 2.4; 95% CI, 1.1-3.0). A combination of *hopQ* type I/*hopQ* type II genotypes were observed in 36 (40.9%) patients and statistical analysis demonstrates that there is a significant association between the simultaneous presence of these two genes (P=0.004).

Discussion

H. pylori cause the most prevalent bacterial infections globally (18). The adherence of *H. pylori* to the gastric mucosa have important roles in the initial colonization and long-term persistence on the gastric mucosa as well as in the intensity of the resulting inflammatory response. Colonization of *H. pylori* usually does not result in clinical consequences but can increase the risk of developing peptic ulcer diseases, gastric adenocarcinoma and lymphoma (19). It has been suggested that specific genotyping-based analysis of *H. pylori* isolates can be useful for predicting post-infection disorders (20).

In the present study, the prevalence of *H. pylori hopQ* type I and type II genotypes was analyzed in patients with different gastrointestinal diseases. The predominant genotype In the Azerbaijan area was the *hopQ* type I genotype found in 52 (59%) cases and this high rate of *hopQ* type I was in contrast to a study, also performed in Iran, that considered the finding of 52% *hopQ* type II genotypes as being high (21). However, the present finding concerning the frequency of *hopQ* type I is consistent with a previous study conducted in the USA (11). Geographical differences in the distribution of type I and type II hopQ alleles have been noted. For example, the majority of *H. pylori* strains isolated in East Asia are cag PAI-positive and contain type I *hopQ* alleles. Type II alleles are commonly identified in *H. pylori* strains isolated in Western countries, but are uncommon among *H. pylori* strains from East Asia (4).

In the present study, the rate of the *hopQ* type II genotype was found most commonly in GC (61.5%). This finding is similar to results obtained in the North of Iran (21). The present findings showed that *hopQ* type I was most predominant in patients with GU, which is in disagreement with the study conducted in the North of Iran (21). To the best of our knowledge, this is the first study on the prevalence of *H. pylori hopQ* alleles, among patient with gastrointestinal disease in the Azerbaijan area, and this is the second study in Iran to determine the prevalence and association of these virulence genes with clinical outcomes. The present findings have shown that the *hopQ* type I and type II genotypes are associated with gastric cancer. The study by Cao and Cover (11) was conducted in the USA and reported that there was a significant association between the carriers of *H. pylori hopQ* type I among the peptic ulcer patients (11). However, Ohno *et al* (22) did not identify an association between the two hopQ alleles and clinical outcomes. Additionally, the combination of *hopQ* type I/*hopQ* type II genotypes were evaluated in the present study in association with the clinical outcomes and statistically significant correlation was identified between these alleles and disease conditions. It appears that these two genes are located in one place and may have a synergistic effect.

The prevalence and association between clinical outcome and the *H. pylori* virulence gene was reported to be different among various countries, regions, ethnicities and patient groups. The differences between studies may be due to geographical area, sample size, studied groups, ethnicity, primer sets, PCR conditions and the variety of bacterial strains. The weakness and limitations of the study firstly were low sample size and the small number of studies performed to obtain a whole evaluation on the prevalence of genes.

If additional studies showed similar results to the present findings, this may lead to improved diagnostic policies for clinicians to fight the present doctrine for gastric diseases. In conclusion, the association between *hopQ* types I and II, and gastric cancer outcomes, may suggest that these genes may be helpful for the prediction of specific-disease risk.

Acknowledgements

The authors are grateful to Dr H. Banazadeh Baghi for the English editing. The study was financially supported by Tabriz University of Medical Sciences.

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